WO 01/53665

PCT/USBL/011V0

plasmon frequency (520 nm), the molar extinction coefficients (x at 520 nm) were calculated for the particles, typically  $4.2 \times 10^8 \, \text{M}^{-1} \, \text{cm}^{-1}$  for  $15.7 \pm 1.2 \, \text{nm}$  diameter particles.

## F. Preparation of Gold Thin Pilms.

- Silicon waters were out into ~10 mm x 6 mm pieces and cleaned with pirants etch solution (4.1 concentrated H<sub>2</sub>SO<sub>4</sub>: 30% H<sub>2</sub>O<sub>2</sub>) for 30 min at 50 °C, then rinsed with copious amounts of water, followed by ethanol. (Warning: piroute each solution reacts violently with organic materials and should be handled with extreme coutton.) Metal was deposited at a rate of 0.2 nm/s using an Edwards Auto 306 evaporator (base pressure of 3 10 x 10<sup>3</sup> millibar) equipped with an Edwards FTM6 quartz crystal microbalance. The exidized sides of the silicon were coated with a Ti adhesion layer of 5 nm, followed by 200 nm of gold.
- O. Preparation of S' Alkythiol Oligosyclestide-Moduled Gold Nanoparticles. Gold parreporticles were modified with fluoresocin-alkylihial oligomedectides by 15 adding freshly deprotected oligonucleotides to squeous nanoparticle solution (particle concentration ~10 mM) to a final oligonucleotide concentration of 3 µM. After 24 hours, the solution was buffered at pH 7 (0.01 M phosphete), and NaCi solution was added (to final concentration of 0.1 M). The solution was allowed to 'age' under these conditions for an additional 40 hours. Excess reagents were then removed by contribugation for 30 20 minutes at 14,010 rpm. Following removal of the supernature, the red oily precipitate was washed twice with 0.3 M NaCl, 10 mM phosphate buffer, pH 7, solution (PBS) by successive contribugation and recispersion, then finally redispersed in fresh buffer solution. Invariably, a small amount (~ 10% as determined by UV-vis spectroscopy) of nanoparticle is discarded with the supernature during the washing procedure. Therefore, 25 final paraparticle concentrations were determined by TEM, ICP-AES, and UV-vis
  - spectroscopy (see above). Extinction coefficients and particle size distributions did not H. Preparation of S. Alkylthiol Olipcountentide-Modified Gold Thin Films.

change significantly as a result of the oligonucleotide modification.

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Silican supposed pole that filters were interested to despections exhalten end buffer objections and file of dispersacional and required firms and buffer conditions as for the pold management. Following dispersacional despection, for filters were stand extensively with 0.3 MF PBB and stored in buffer includes. Once the war responsed on one distinct buffer in the contract of the war responsed on the filter of the contract of the standard production and the first indexes, which the modified DFA did not admind appreciately so have allicon oxide nurthern that were rised way DFBS.

I. Quantitation of Alkylthiol-Olisconucleotides Loaded on Nanoparticles. Mercapicetisand (MB) was added (final concentration 12 mM) to fluorophore-10 Inheled oligonoclectide modified nanoparticles or thin films in 0.3 M PBS, to displace the oligomolectides. After 18 hours at room temperature with intermittent shaking, the solutions containing displaced oligonucleotides were separated from the gold by either centrifugation of the gold nenoparticles, or by removal of the gold thin film. Aliquots of the supernatest were diluted two-fold by addition of 0.3 M PBS, pH 7. Care was taken to 15 keep the pH and fonic strength of the sample and calibration standard solutions the same for all measurements due to the sensitivity of the optical proporties of fluorescein to these conditions (Zhao et al., Spectrochimica Acta 45A:1113-1116 (1989)). The fluorescence maxima (measured at 520 mm) were converted to molar concentrations of the flaorescentalkylthiol modified oligonucleotide by interpolation from a standard linear calibration 20 curve. Standard curves were proposed with known concentrations of fluorephore-labeled objectualentides using identical buffer and salt concentrations. Finally, the average number of eligonucleotides per particle was obtained by dividing the measured oligenus lectide malar concentration by the original Au ranoparticle concentration. Normalized surface coverage values were then culturated by dividing by the estimated 25 particle surface area (useuming spherical particles) in the raneparticle solution. The assumption of roundness is based on a calculated average roundness factor of 0.93. Roundness factor is computed as: (4 x pi x Area)/(perimeter x 2) taken from Baxes, Gregory, Digital Image Processing, p. 157 (1994)

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WO 03/51/655

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1. Compliation of the Herbeitson Travels Statistical Examist.
To decimine the neithyrol settlends (injectuationals for by hydratation, these phone beheld oligenuclosides, which were complicated by the behalded oligenuclosides (117), were reacted with eligenuclosides and oligenuclosides (117), were reacted with eligenuclosides and oligenuclosides (118) and the hydrodization conditions (2) all completes considerable superioristics (3.3 M FSSs, [91, 23 hs.). Non-lyndridized disposulosides were reasoned from the pile by rinting twice with futures and large activated other. Then, the throughter beheld alignmentation were dephylational by subdition of Mod (first and construction)—30 Aug 11 ± 12, 48, 79 hourse spenders of the obtains constraints
10 that 127 has the mosphricis deatheast by cereinfugation, and committation of the extension of M MSC (first and modernized only hydrides degreeabout and construction)—30 of MSC (first and construction)—30 of MSC (first and constructions)—30 of MSC (first and constructions)—40 of MSC (f

#### K. Quantitation of Surface Coverage and Hybridization

15 Climat ráskilized gold canoporticles were finacinalisad vili. (Dare finorezoeio-suodilized adjetnio IDAA (ISI,GEN)p-6-COCA TIT-GAG-DAT-(CR),PF [SEQ ID NOC50]). Sirtic correspet studias were then per formod by treatedyl friendig wary non eleminodated oligometicoloides, followed by remained of the fluorephote-blotted oligometicoloides, fluored by remained of the fluorephote-blotted oligometicoloides from the gold améter, and quasitionio of oligometeoloide concentration wing fluorescences spectroscopy (se described aboves).

Reserved of all the disputational for the plan states and schemous memoral aged adaptional from from the children in critical the destingles counted converged ages by forcements for several reasons. First, the furnitures in critical for the children of the children of the children of the children of the children converge research to be could DNA in difficulty operated as a resolution of the converge consonner congre research (as forced as the children congre research of the children of the children congregate children of the children consonner conson

WO 03/51665

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significant unouse of light between 200 are and 530 am, so their presence in solution during florecontence measurements sets as a filter and diministrice the available excelsive energy, as well as the intensity of essisted reliation. The gold surface plasmon band at \$20 am falls at the emission maximum of floreconies.

3 Merceptonium (ACP) was used to replief (slight) for auchies bound originactication by an exchange resident, in causine the distillurant barbox, objectoristic to unclide the acceptance barbox, objectoristic modified acceptance are foresteron acceptance. The intensity of foresterons acceptance to ME (12 mM) for increasing people of this prior is constituting against and foresterons acceptance. The intensity of foresterons acceptance with the sealing of interpreting in the seal of intensity of the intensity of the intensity of intensity of intensity of the inten

The average oligonocleotide surface coverage of skly/thiof-modified 12mer oligonocleotide (SLEP) on polit amountained was 4x 1 providers (everage of ten independent measurements of the same). Per 15.7 ± 12 an industrye proteinel, side corresponds to except 159 thiol-bound 12mer transfer, but one to examine the corresponds to except 159 thiol-bound 12mer transfer per gold particle. Dospite slight 10 purples discontinuous contractions of the contraction of the contra

In order to worly fast this enthud is useful for polyticing accusate disposencedood acutes covernage, with was sell to depicted proposence delated disposencedood from good this flour, and the surface coverage data was compared with experiments aimed at 25 gentle; printing their delated part with different techniques. In his near experiments, get that flows were subjected to a suntile edisposenced terminolisation and May depletement procedures as the other subjected and an united techniques for modification of MA depletement procedures as the other stabilized gold an acceptation (see a lower). The eligenseatedness depletement remains coverne for the gold and acceptation (see a lower) and the surface and the subject access to the subject and the surface coverne for the gold and acceptation (see a lower).

**РСТИВИЛИТУ** 

for gold nunoparticles. This suggests a similar rate of displacement for the thin filent, even though the typical surface coverage values measured for these films were asserted slower than the oligonomic point coverages on gold annoparticles. Importantly, the oligonucleotide surface coverages on gold thin films measured by our technique (18.2.3

- organizacione sea tana circago organizamente instruccion y our suspensione (1 e. 2.5 possiblem) fill silvi within the trange of previously reproted converges on oligometeolethe to Elem (10 prosilvim) for a 25 base oligometeolethe on gold electrodes determined using electroschemistry or surface plasmon reseasors spectoropy (SPRS) (Stotle et al., dest., Celem. 7(%-67-67) (1998)). Differente in auchines coverages are expected due to different oligometeolethe requience makings, as well as full preparation methods.
- The course of hybridization of complementary (Incorphoca-balled oligonacidental (CPP) to incorporation with derived-board Linear Signature disease measured an described above. Brilling, SEP coedified inserporation were exposed to 2PF at a consensation of 2 pM for 5th wears who hybridization continuous CPM MPSS, pRT 7 and these crossed extraversity with buffer solution. April, in 1-was reconstany to 2PF and 2PF and
- (approximate) & dupletes per 15.7 mm periods, the average number of deplotous per partials was compared by multiplying the memberal by Polishing states overage to periods was compared by Tamilla, polish periods periods and period by Tamilla, polish periods period and periods and period by Tamilla, a polish period period and periods and periods and period and periods are deposed to the temperature of the period and period and period and periods were acquested to the temperature that period and periods are consistent of the period and periods and periods are acquested to the period and and periods are acquested to the periods and and periods are acquested to the periods and and periods are acquested to the periods and periods are acquested to the periods and periods and periods are acquested to the periods and periods are acquested as a period and periods ar
  - non-specifically adjoorhood oligocunolectides on the nanoparticities was determined to be do the order of 0.1 providen? An anakagous procedure was used to measure byticalization to \$12F modified gold thin fillms in order to compare the hybridization results to reported values on gold electrodes. The degree of hybridization, 6 ± 2 pmol/om?, was consistent

PCDUSHI/01391

with hybridization reported for mixed base 25mer on an gold electrode (2-6 pmol/cm²) (Steel et al., Anal. Chan. 10:4670-4677 (1998)).

Buffer coverage so the judicidation value of the SEPETAPE "years for both amagestriction and finite are commanded to Table 7. The most stating smalls in the 5 in the product of the SEPETAPE OF THE SEPETAPE

L Effect of Oilsonatorial Source on Burkes Consess and Ethniciation.

Although the lips coverage of the 25 Telegometeration is a chargeon in terms of assepticities and Illiation, the two hybridization (Efficiency prompted as to device a means of fectorating entire congestion sound the hybridizing requirement.

15 Oilgeanatorial Charry were synthesized barding 20 GA spaces requirement to between the absyliation proposed the descriptor II has tensopiolism requirement. This stronger was closers based on the soundprofes and the same of the missage centre are strengted hybridization because of weak interestions between the relivoyment on the region of the gold methods, and with interment device ordering, and of you 15 Tens dismontive the region of the proposal pr

(1999) will had be a fig. with a greater few values as compared with a 60m femmed from the same 12m decolory beam of a see action.

While the nucleon density of single-stunded 8.A<sub>2</sub>1.07 strands (15.4 e/moblem).

22 was been than that of 1272 OF 41 possitions), the particles soudified with a 32-mm. on single beliefacted into the confidence of the seed of the seed

WO 01/51065

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original S12FV12F \* system, Table 7.

- M. Effect of Electrotic Concentration Device Officerowicalistic Absolutes
  In weeking with the SIDF requesters all single few was for sold to extend in
  obtaining make of ignorecturable anotherin composition for Enzage 19. The gold
  omnopatition modified with SIDF is post water two fasgester irrecently for form
  block precipitate upon confidenting, which those spel is salt resisted aggregation when
  constituting with the higher cologoscological two corrects and by all whose higher cologoscological two corrects and by all whose higher cologoscological two corrects and by all whose the contentiation of the solid cologoscological two contentiations and the cologoscological two contentiations and the cologoscological two contents and the cologoscological two contents and the cologoscological two cologoscological two cologoscological cologoscological two based on the cologoscological cologoscological two based on the cologoscological col
- 15 It is expected to make the design of the comparison of the comp
  - offentively acreems charge repulsion between unsighboring oligonucleotides, as well as, attraction between the polyanizatio oligonucleotide and the positively charged gold surface. This allows more oligonucleotides to bind to the nanoparticle surface, thereby

WO 0150665

PETTANDIAN 190

increasing oligonucleatide surface coverage.

N. Effers of Olimenschedigk Space Sequence on Surface Coverage.
In octar to examina how the requessor of the spaces affects oligonucleotide
coverage on An assopaticities, Risensected-worlfield 2-mer attends, with 20 AA and 20
5 of Tapaces instetted between a 3 yearys lithal and the Risensecisis-labeled 12-mer requested,
when perspects if the most notable seafes of surface coverage and shyddistantion studies of

www.popend. The most notified seated of earlies coverage and hybeflaterists todded as temporalistic source of the high seated of the control of the coverage and hybeflaterists to the 20 de to general colors with the 20 de 10 percent (24 ± pentioles). The comparison to the 20 de topes (24 ± pentioles). The monther of hybeflaterist is not in vac comparable, although the presentage of weither bound errords with hybeflaterist was bown for \$73,47 large managements (27 %) than the \$4.0,27 managements (27 %). These results suggest that of this objection between two interests may received large with the seate of the side percentage of the 30 december of the 3

15 while 20dA spacer regreents block gold sites by fying flat on the particle surface.
O. Effect of Conductived Diluent Observations.

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hybridization and protecting the surface from non-specific adsorption.

Nanoparticles were modified using solutions containing different recognition

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stread (SA<sub>20</sub>12F) to dilutes (SA<sub>20</sub>) strand molar ratios. The resulting particles were analyzed by the fluorescence method described above to determine the SA<sub>20</sub>12F surface density, and then tested for hybridization efficiency with 12F.

The SA<sub>10</sub>12F within deathy homesed linearly with respect to the proportion of  $SA_{10}170 \times SA_{10}$  in the deposition solution, Figure 90. This is an interesting result because in suggests that the rote of  $SA_{10}170 \times SA_{10}$  is the charge that the cross of  $SA_{10}170 \times SA_{10}$  such chief to the canaparticles reflected dut of the solution. This result is constant to what is normally seen for inclusion about a first of Technicalization folicy was to solidity and which in legally days omitted who is educated to the first of  $A_{10} \times SA_{10} \times$ 

The amount of complementary 12°F objectsclosside which hybridated to each different sample also increased linearly with increasing SA<sub>2</sub>12°F authors ownerspa. Pigers 31. The fact that is relationable to self-defined indicates that his possible to preclide and control the extent of hybridization of the conseportable-obligamenholds energipters. 15 This registers that hybridization of 11°F becomes some difficult at higher SA<sub>2</sub>12°F coverages, which is non Richly a result of source course of fifting at higher SA<sub>2</sub>12°F coverages, which is non Richly a result of source course graph which is non Richly a result of source course graph of the source course graphs of the source course graphs of the source course graphs of the source course graphs.

# between oligonucleotides. P. Summary.

This mody has there that it is important to active as behavior between obligational decreasing high money to entitle the numperiodical to which they are united, by so we enough to that is high percentage of the tumbor is exceedable for hybridization with objectness decide in solution. This has been active only a digital condition decrease (injurational decided in solution) to the acceptation of the enough of the production of the acceptance of the acceptance

escensisment interactions, and consented amount intuities to opposite they constitute awarding member of hybridization and events for each interaction. It has also been shown that the nature of the softer (special) sequence influences the number of oligonactionide atmode leaded onto gold resopratisches. This work has importent implications regarding

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understanding intornolises between oligonucleotides and nanoparticles, as well as optimizing the sensitivity of sanoparticle-oligonucleotide detection methods.

#### \*\*\*\*\*

Single strand surface coverage and corresponding hybridized surface coverages for gold this films and gold nanoparboles. Comparison between 512F and SA<sub>W</sub>12F surface coverage and hybridization. This mostled organizationalists were statistical to the gold from 3 plut agreement professional and to 0.1 M bid CI M biddle-like statistics which are professional in 3 M DSR and 2

Oligonucieosida Pair	Burlace Goverage (pmol/pm²)	Hybridization Coverage (protion <sup>2</sup> )	% Hybridization Efficiency
	Aun	anoperticles	
\$12F/12F	34 ± 1	1.3 ± 0.2	- 4%
SA <sub>W</sub> 12F/12F*	15 ± 4	6.6 ± 0.2	- 44%
	A	thin fras	
612F/12F'	18±3	6±2	- 33%

WO 91/51/65

PCT/USBL/#1198

#### TABLE 8

Buffer conditions duning subscription of alkythiol DNA	Surface Coverage (prod/cm²)	Hytxidization Coverage (pmol/cm²)	Hybridization Efficiency (%)
H <sub>0</sub> O	7.9 = 0.2	-*	
0.1 M NaCL 10 mM phosphale	15 ± 4	6.6 ± 0.2	-44
1.0 M NaCl, 10 mM phosphate	20±2	6.6 ± 0.2	-33

#### TABLE 9

nce on surface coverage and hybridization efficiency.

Oligonucleolide Pair	Surface Coverage (prooform <sup>2</sup> )	Hybridizaţion Coverage (pmollom <sup>*</sup> )	Hybridization Efficiency (%)
63YA <sub>00</sub> 12F / 3'12F	24±1	9±2	-38
\$37 <sub>m</sub> 12F / 3'12F	35 ± 1	12 ± 1	-34

312F = 5'-ATC-CTG-AAT-GCG-F [SEQ ID NO:54]

#### Example 19: Good Chip Assay

An ultrasolactive and ultrasensitive method for analyzing combinatorial DNA arrays using oligorucleotide-functionalized gold nanoparticles is described in this 25 example. An unusually narrow temperature range for thermal dissociation of nanoparticle-target complexes permits the discrimination of a given oligogracheolide sequence from targets with single aucleotide mismatches with extraordinary selectivity.

132

In addition, when coupled with signal amplification method based on unreperticlecatalyzed reduction of silver(I), the sensitivity of this nanoparticle array detection system PCTYCSOL/01394

exceeds that of the analogous, conventional fluorephore system by two orders of

Sequence-selective DNA detection has become increasingly important as scientists unravel the genetic basis of disease and use this new information to improve 5 medical diagnosis and treatment. Community used heterogeneous DNA sequence detection systems, such as Southern blots and combinatorial DNA chips, rely on the specific hybridization of surface-board, single-strand capture oligonactentides complementary to target DNAs. Both the specificity and sensitivity of these assays are dependent upon the dissociation properties of capture strands hybridized to perfectlymatched and mismatched targets. As described helow, it has surprisingly been discovered that a single type of nanoparticles hybridized to a substrate exhibits a melting profile that is substantially shaper than both the analogous fluorophore-based system and unlabeled DNA. Moreover, the molting temperature for the nanoparticle duplex is 11 degrees higher than for the analogous fluorophore system with identical sequences. 15 These two observations, combined with the development of a quantitative signal amplification method based upon remonsticle catalyzed reduction of silver(I), have allowed the development of a new chip-based detection system for DNA that has singlebase automatch selectivity and a sensitivity that is two orders of magnitude more sensitive than the conventional analogous fluorescence-based assays.

20 Gold ansequentine () I am alliametry baving oligosustronies stacked to them proposed as described in Energia I have used to indicate the generate of a phrediative DNA sequence by Indiand to a temperore on Aventa in a time component moderial away throat (see Piger 20). In a typical experiment, a shortest was phistomed by floatisecalities and price aminous good information control of the second 20 posses oligonulesceless as described in Exemple 10. This entend we as used to generate sinker floatisecalities and described in Exemple 10. This entend we are used to premise of multiple types of oligonucleosities over their cells are away of multiple types of oligonucleosities worker that consumers in informatives. Management in America microlar registered as moderal to student to the outputed 50 enter specification of the control of the contro

PCTWSBUDD 150

oliganucleatide targets (based on the anthrax protective antigen sequence) were then cohybridized to these substrates (see Figure 32). Therefore, the presence of nanoparticles at the surface indicated the detection of a particular 30-base sequence. At high target concentrations (≥ 1 nM), the high density of hybridized nanoparticles on the surface 5 made the surface appear light pink (see Figure 33). At lower target concentrations, attached nanoparticles could not be visualized with the naked eye (although they could be imaged by field-amission scanning electron microscopy). In order to facilitate the visualization of nanoparticles hybridized to the substrate surface, a signal amplification method in which allver icus are estalytically reduced by hydroquinone to form silver 10 metal on the slide surface was employed. Although this method has been used for enlargement of protein- and antibody-conjugated gold canoparticles in histochemical microscopy studies (Hacker, in Colloidal Gold: Principles, Methods, and Applications, M. A. Hayat, Ed. (Academic Press, San Diego, 1989), vol. 1, chap. 10; Zehbo et al., An. J. Parhol. 150, 1553 (1997)) its use in quantitative DNA hybridization assays is novel 15 (Tombinson et al., deal. Blockers, 171:217 (1988)). Not only did this method allow very low surface coverages of nanoparticle probes to be visualized by a simple flatbed scanner or the naked eye (Figure 33), it also permitted quantification of target hybridization based on the optical density of the stained area (Figure 34). Significantly, in the absence of the target, or in the presence of noncomplementary target, no staining of the surface was 20 observed, demonstrating that potther paraspecific binding of nanoparticles to the parface, nor nonspecific sliver staining, occurs. This result is an extraordinary feature of these unoparticle-oligonuolectide conjugates which coabtes ultra-sensitive and -selective detection of rupleic sizes.

It has been determined that the unique by-indiffusion properties of diligomented obfementiculation ammonificates of the present invention can be firstfuer used to improve, the selectivity of combinatorial oilgomented interps (or 'grant oligin') (Todan, Science 177, 300 (1979)). The relative ratio of target hybridized its different demands of an oligomedicability rarry will determine the accuracy of the acting in determining the target.

PCTP/CSUG/RI 199

sequence; this ratio is dependent upon the hybridization properties of the duplex formed between different capture strands used the DNA ranges. Remarkelly, three hybridization properties are disamatically improved by the use of nacoparticlo labels instead of fluocophore labels. As shown in Figure 15, the dobybridization of nanopastic-labeled

- 5 unegate from a reface-bound captors streads was much more sociality to temperature that the off discreption-labeled targets with identical separation. While the flumesphere behinded them after leaves transfer with the provided larger same from the very two years larger same years; (first decivalse TVV)Def –16°C), below and insight in decivalse TVVDef –16°C), in leaves already of the decivalse TVVDef –16°C), in leaves already of the discrept market and with more about joint decivalse TVVDef –3°C). It was satisfied that there are already of the discrept discreption of the discreption of the discrept discreption of the discreption o
- sacjynis, which is usually effected by a post-hybridization stringcrey wath. Indeed, the ratio of fazesh hybridized for complementary surface probes to that hybridized to emissantiched probes office a stringcrey wash as a sportice temperature (represented by the residence liberie in Figure 43) is much higher with nampearise labels than filesceptives.

  15 labels. This is doubt stransite to higher existedity in child protection formats. In addition,
- 15 inhels. This aboutd translate to higher estectivity in chip detection formats. In addition transparticle labels should increase array sensitivity by raising the melting temperature (T<sub>n</sub>) of surfuce despicaces, which lowers the critical concentration below which duplexes specificacously melt as room temperature.
- In creter to evaluate the differences of nanoparticles as colorisates indicators

  Be eligomothotic energy, set rollays were product with a synthetic target and labeled with

  the discontrols and entemportic indicators. The test survey and eligomothotic target

  were followated according to published protocols (Gion et al., Nocl. Acids Bez., 225455

  (1994); emps of 175 jun dissumer epots aspected by 795 im mere pointeed using a

  Gamelia Microsyne 414 followarpset, Aurys contained four destinates congregations.
- 25 to the each of the four possible successides (N) at position 8 of the target (see Figure 32). The synthetic target and either fluorescene labeled or nanoparistic-beholed probes were hybridized supervise to arrays in hybridization buffer, and each step was followed with a stringency buffer wash at 33 °C. First, 20 µL of a 1 mM solution of synthetic target in 2.

WO 01/51/45

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X78(0) 0.1 M/CCI, 0 mod X10(4)(CI, 0 mod X10(4)(CI, 0 mod X10(4))

may for it down or commergenate in a legislication calabore (loss B-14 and Core
Well YCZO), and fine wealsed it 370° with claim X78 bits Borlie. Note, X20 pile 4 mills
poli solution of collegenocitation demonstrates god temperation in 5 x 785° with
5 bylinication in the curry for 4 shows a room temperature in a final bylinification observed.
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5 bylinication in the curry for 4 shows a room temperature in a final bylinification observed in the curry for the collection of the control of the room of the control of the collection of the control of the collection of the study in regard of the final final collection of the control of the final final collection of the study in regard with a finished of the collection of the study in regard with a finished considerable of the collection of the study in regard with a finished considerable of the study in regard of the finished collection of the study in regard of the finished collection of the study in regard of the finished collection of the study in regard of the finished collection of the study in regard of the finished collection of the study in regard of the finished collection of the study in regard of the finished collection of the study in regard of the finished collection of the study in regard of the finished collection of the study in regard of the finished collection of the study in regard of the study in regard of the finished collection of the study in regard of the finished collection of the study in regard of the finished collection of the study in regard of the finished collection of the study in regard of the study in reg

scauner or even the naked eve. Arrays challenged with the model target and nanoparticle-labeled probes and stained with the silver solution olearly exhibited highly selective hybridization to complementary array elements (Figure 36A). Redundant spots of the same capture 15 sequence showed reproducible and consistent hybridization signal. No beckground adsorption by nanoporticles or silver stain was observed; the image greyscale value reported by the flatbed scouner is the same as that observed for a clear microscope slide. The darker spats corresponding to admine at position 8 (N=A) indicate that oligenucleotide target bybridized preferentially to perfectly complementary capture 20 strands over mismatched ones, by a greater than 3:1 ratio. In addition, integrated greyscale values for each set of spots follows the predicted stability of the Watson-Crick base pairs, A:T > G:T > C:T > T:T (Allowi et al., Biochemistry 34, 10581, (1988)). Normally, G:T mismatches are particularly difficult to discriminate from A:T complements (Stiki et al., in Musasion Detection, Cotton et al., eds. (Oxfood University 25 Paress, Oxford, 1998), chap. 7; S. Bouta et al., Nucl. Acids Res. 15, 797 (1987)), and the distinction of faces two army elements demonstrates the remarkable resolving power of nanoparticle labels in single carcleotide reismatch detection. The selectivity of the assocparticle-based arrays was higher than that of the Eucrophore-indicated arrays, Figure

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36B, fluorophore labels provided only 2:1 selectivity for admine at position 8.

The easyst utilities associated before protein resignificantly more emission to the final frame/one-holded points. Politification, significant the Ho-A columnum at import consistent one in two as 50 Me, on the a spherituation is low as 50 Me, on the a spherituation is consistent on satisfied and oppiny, this represents a demand is consistent on satisfied your common SpACOS throughout-belood on strong the while—I plut or general segant consistention in the yearth presign. The higher methics associated in satisfied for competition-length competition in the process of the satisfied in students.

umdoubtedly contribute to array sensitivity. The greater stability of the 10 probettaged/suptime of ligocoubeoide complex in the case of the pure-particle system as compared with the fluorophire system prevumably results in less target and probe lost during washing steps.

Colorimetrio, nanopariche labeling of combinatantal oligozostootide zmays will be methic in opplications socks at single methodide polyamphism analysis, where single intermetal treations, metablishing, cost moder of use are important factors. Moreover, the soundariest yet of this system, which has yet to be testilly optimized, yolins towards a potential method for descenting objector-locife largest without the need for targest amplification necknown seek as a polyameter social nection.

#### 20 Example 20: Nanoparticle Structures

The reversible assembly of supremolecular layered gold nanoparticle structures only glass supports, meditated by hybridized DNA linkers, is described. Layere of eligencelocitie-functionalized assoparticles were successively statehold to eligencelocitie-functionalized glass substrates in the presence of a complementary DNA

25 linker The unique recognition properties of DNA allow the nanoparticle structures to be assembled educatively in the presence of the complementary factor. In addition, the structures can be asteroibled and dissonabled in response to external retunit which mediate hybridization of the licking duplex DNA, clouding subdicts extensively.

of biodetection.

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and lonic strength. In addition to offering a very schoolive and controlled way of building sunspecticle based architectures can a rolled support, this system allows one to study the factors that influence both the optical and mothing properties of exceptionarticle network streamers limited with DNA.

- 6 O'Deen have democutanted bow hidusectional organic molecules (Giffins et al., Adv. Mater. 13,177 (1999); Brant et al., Lougnau's 14-539 (1998); Brant et al., Lougnau's 14-539 (1998); Grahe et al., J. Am. Crieni. Soc. 153 (1998); Grahe et al., J. Am. Crieni. Soc. (144) (1999); Presente et al., J. Societa (24) (144) (1990); Presente et al., Crieni. Mater. 14, August. Chem. Int. Ed. Engl. 39-131 (2000); Matchiston et al., Chem. Mater. 16-1214 (1998)) or polyelectrolyse (Stochaff et al., J.
- 10. Am. Chem. Soc. 120 (1997) (1998), Standerf et al., A. Chem. Col. 8137 (1998), Ballestin et al., Science 277:1617 (1997), Ballestin et al., Chem. Chem. 277:1617 (1997), Ballestin et al., Chem. 278:1617 (1997) and seat and section of the chem. 278:1617 (1997) and seat and seat and content and content
- enumg programs on ungeninement we assure on a reminion as introduced an introduced and assure than kinetic structure. In delicition beyonding a new and power all antificial for controlling the growth of assurantificial-based architectures from solid substance, this strategy also allows one one or-wintow the substanding between appropriate pages and both meeting and optical proportion of aggregate DNA interfacided structures. An understanding of these two physical parameters and their relationships beautified auditoriated and interfaced and
- The objected entitle functionations. It is medianester and of unemposities used to combute the multi-layer assemblies were prepared as discorbent in Examples 1 and 2. The assemptation had "Sharazanthind-copyed eligentelectrick 1;" "SECTALNOOPO," OCIOATTICAGONT'S [SEQ ID NO-09] and "proposenticle-topped eligentelectric 2" SECTALNOOPO," OF ACTICAGONT CONTROLL TO SEQ ID NO-09) until and on them to for "SECTALNOOPO," OF ACTICAGONT COTT SEQ ID NO-09) until and on them to

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yield unnoparticles a and 8, respectively (see Figure 37). Glass slides were functionalised with 12-mer oligonucleotide 2 as described in Example 10. To build nanogamicle layers, the substrates were first immersed in a 10 nM solution of 24-mer linker 3 (5"-TACGAGTTGAGAATCCTGAATGCG-3" [SEQ ID NO:60]) and allowed to 5 hybridize with It for 4 hours at room temperature (see Figure 37). The substrates were washed with clean buffer solution, and then hybridized with a 2  $\alpha$ M solution of particle  $\alpha$ for 4 hours at room temperature to attach the first nanoparticle layer. A second nanoparticle layer could be attached to the first one by similarly exposing the surface to solutions of linker 3 and nanoparticle &. These hybridization steps could be repeated to 10 attack multiple, alternating layous of nanoparticles  $\alpha$  and  $\delta$ , each layer connected to the previous one by linker 3. In the absence of linker, or in the presence of noncomplementary oligomelectide, no hybridization of nanoparticles to the surface was observed. In addition, multilayer assembly was only observed under conditions which promoted the hybridization of the DNA linkers: neutral pH, moderate salt somewhation 15 (> 0.05 M NaCi), and a lemperature below the duplex malting temperature (Tm). Each hybridized unnoparticle layer imparted a deeper red color to the substrate, and after ten hybridized layers, the supporting glass stide appeared reflective and gold in color. Transmission UV-vis spectroscopy of the substrate was used to menitor the successive hybridization of nanoparticle layers to the surface, Figure 38A. The low 20 absorbance of the initial nanoparticle layer suggests that it seeded the formation of further layers, which showed a near linear increase in the intensity of the pleamon band with each additional layer (for each successive nanoparticle layer formation, no additional absorbance was observed on exposure for longer times or to higher concentrations of either linker 3 or nanaparticle solution). The linearity of the absorbance increase after the

25 generation of the initial ransoparticle layer indicates that the surface was substant with hybridized susquestibles with such successive application, Figure 388. This is supported by field-emission scenaring electron microscope (FF-SEM) images of one (Figure 39A) and love (Figure 39D) sunoparticle layers on a surface, which show low susquestible.

WO 03/51665

PCDWMA119

coverage with one layer, but near complete coverage with two layers. The  $\lambda_{\rm max}$  of the plasmon hand for the multitryer assemblies shifts go more than 10 nm, even after 5 tayers. The direction of this shift is consistent with other experimental (Grabar et al., J. Am, Chem. Soc. 118:1148 (1996)) and theoretical (Quinter et al., Surf. Sci. 172:557 5 (1986); Yang et al., J. Chem. Phys. 103:869 (1995)) treatments of gold nanoparticle aggregates. However, the magnitude of the shift is small compared to that previously observed for suspensions of olivensuclootide-linked sold renonanticle networks, which show  $\lambda_{max} > 570$  nm (see previous examples). This suggests that many more linked namograticles --- perhaps hondreds or thousands --- are required to produce the dramatic 10 color change from red to blue observed for gold nanoparticle-based oligonacleotide probes. (Storhoff et al., J. Am. Chem. Soc. 120:1959 (1998); Storhoff et al., J. Cluster Sci. 8:179 (1997); Elgharuan et al., Science 277:1078 (1997); Mirkin et al., Manare 382:607 (1996).). Surface plasmon shifts for aggregated gold nanoparticles have been abown to be highly dependent on interparticle distance (Quinten et al., Surf. Sci. 172:557 (1986); 15 Storhoff et al., J. Am. Chem. Soc., in press), and the large distances provided by oligonucleotide linkers (8.2 cm for this system)) significantly reduce the progressive

The describion programme of the numbried competition multilayous were highly deposited upon the number of layous. When the multilays-record substrates were semigented in huffer ordinion and the temperature sized above the 7-, of the linking eigenmentation (2007, the exceptionfied assistant and interesting things of accessing the semigented passes of the linking passes of the concentration of the linking interesting the semigented and concentration of the linking expension of 10 M I N or 3 M or described the and concentration of the linking interesting the semigented in the linking IRAN. The meditation are deliverable such expension of the linking IRAN. The meditation are deliverable such that the glass substrates (a.g. three cycles were described with 10 described in reversible anapprecise duals the hydrogen and the substrated of the semigration duals the hydrogen than the substrated of the semigration and the system of the semigration and the semigration and the system of the semigration and the system of the semigration and the system of the semigration and the semigration and the system of the semigration and the system of the semigration and the semigration and the system of the semigration and the system of the system o

effect of nanoparticle aggregation on the gold surface plasmon band.

Significantly, while all of the surface bound nanonarticle assemblies dissociated

WO ENTHERS

PCT/USDIAL 100

stern not. T<sub>e</sub>. of the linking originameloution, the chargest or done transition depended on the size of the approach aggregate, Figure 1902. Superringly, the Missionation of the first canoposition by the first one behavior. Collidar is a statistical by the Figure 1902, 1979.03. Operating the Missionation of the first canoposition by the first first canoposition between a "Old blank was being the that of the first confidence foundation association in competition to enclaim figure 1902. As more associated layers were highlighted to the sections, the mediag invasion of the oligoposition fields associated to be consistent of the oligoposition fields associated to be consistent of the oligoposition device as supervised to the first devictive 2 = 1°C, until the method data of the first permitted to strong the supervised the other lates of the supervised the other lates of the supervised to the data of the first permitted to whom the visit of the supervised to device the consistency of the supervised to device the consistence of the supervised to device the supervised to device the consistence of the supervised to device the consistence of the supervised to device the supervised

#### 15 Example 21: Electrical Properties of Gold Nanoparticle Assemblies

Electron tramper (devengh, DMA has been one of the most intrestig debend subjects in chemistry over the pair person, (Collige or A., Science 281375-311 (1999); Turn or et al., 2013 2-201-209 (1999); Lowis or et al., 2013 2-115-221 (1990), Robots, M. Alanzer 397-480-481 (1999); Debats on et al., 24 no. Chem. Soc. 178-315-4166 (1995) Obstated on et al., 24 no. Chem. Soc. 178-315-4166 (1995) Obstated on et al., 24 no. Chem. Soc. 178-315-4166 (1995) Obstated on et al., 24 no. Chem. Soc. 178-315-4166 (1995) Obstated on et al., 24 no. Chem. Soc. 178-315-4166 (1995) Obstated on et al., 24 no. Chem. Soc. 178-315-4166 (1995) Obstated on et al., 24 no. Chem. Soc. 178-315-4166 (1995) Obstated on et al., 24 no. Chem. Soc. 178-315-4166 (1995) Obstated on et al., 24 no. Chem. Soc. 178-315-4166 (1995) Obstated on et al., 24 no. Chem. Soc. 178-315-4166 (1995) Obstated on et al., 24 no. Chem. Soc. 178-315-4166 (1995) Obstated on et al., 24 no. Chem. Soc. 178-315-4166 (1995) Obstated on et al., 24 no. Chem. Soc. 178-315-4166 (1995) Obstated on et al., 24 no. Chem. Soc. 178-315-4166 (1995) Obstated on et al., 24 no. Chem. Soc. 178-315-4166 (1995) Obstated on et al., 24 no. Chem. Soc. 178-315-4166 (1995) Obstated on et al., 24 no. Chem. Soc. 178-315-4166 (1995) Obstated on et al., 24 no. Chem. Soc. 178-315-4166 (1995) Obstated on et al., 24 no. Chem. Soc. 178-315-4166 (1995) Obstated on et al., 24 no. Chem. Soc. 178-315-4166 (1995) Obstated on et al., 24 no. Chem. Soc. 178-315-4166 (1995) Obstated on et al., 24 no. Chem. Soc. 178-315-4166 (1995) Obstated on et al., 24 no. Chem. Soc. 178-315-4166 (1995) Obstated on et al., 24 no. Chem. Soc. 178-315-4166 (1995) Obstated on et al., 24 no. Chem. Soc. 178-315-4166 (1995) Obstated on et al., 24 no. Chem. Soc. 178-315-4166 (1995) Obstated on et al., 24 no. Chem. Soc. 178-315-4166 (1995) Obstated on et al., 24 no. Chem. Soc. 178-315-4166 (1995) Obstated on et al., 24 no. Chem. Soc. 178-315-4166 (1995) Obstated on et al., 24 no. Chem. Soc. 178-315-4166 (1995) Obstated on et al., 24 no. Che

properties (i.e., small aggregates can give sharp transitions but still not change color).

be an interlator.

In a securingly disparate field of thirdy, a great deal of effort has born devoted to camining the electrical proposition of nanoparticle based materials. (Ternill et al., J. Am. Chem Soc. 1371:12374:12348 (1995); Brant et al., Adv. Mater. 7:795-797 (1995); Betholl

25 et al., J. Electroanel. Chem. 469:137-143 (1996); Mexick et al., Chem. Moter. 9:1469-1501 (1997); Brass et al., Language 14:7425-5429 (1995); Collier et al., Science 277:1978-1981 (1997). Indeed, enany groups have explored verys to assemble assoparticles into two- and throe-dimensional betworks and have investigated the

PC77/QS01/01 150

sloceronic properties of such greatures. However, virtually mothing is known about the electrical properties of nanoparticle-based materials linked with DNA.

For the first time, in this study, the electrical properties of gold sanoparticle

assemblies, formad with different length DNA internateach have been exauthor. As a down below, that shybid isospan anomation behaves a exemption of the an excent conduction, regardless of adjustmentation in principle and the principle and a chemically specified another in principle and the principle

At the heart of this lates the following questions: Cut neceptivities amendate to QNA mill recollective and following the All meteroscapes, which the hearthy found on each particle, Oderic, R. C. Agustanescap Persparenced Memperature Assembly Golder QNA. Thesis 76. D., Northerstein (1997) are as insudating shells? The conductivities of the constraint is a rathesis of temperature, objective the integral partial. The conductivities of the constraint is a rathesis of temperature, objective were extensived. The DNA finished succeptivities speciations were elementative that the conductivities of the ministen asseming element ministency (FGAM), quadrature sentil suggest every scartning (GAMS) experiments, themail densiratoring profiles, and UN-via speciatorings.

In a typical superiment (peo Figure 40), climto-stabilized 13 mm gold and point interest point (in the property of the condition with 3 and 5 information apport 12 mm colligation (in SM (CH<sub>2</sub>)),(O)OO<sup>2</sup> (A-ATGOCTO-ACCET 5' (SEQ D) NO-59)) mml 2 (5' SII (CH<sub>2</sub>),OOO<sup>2</sup>)O-COCATTCAGOAT 3' (SEQ D) NO-59) mml 2 (5' SII (CH<sub>2</sub>),OOO<sup>2</sup>)O-COCATTCAGOAT 3' (SEQ D) NO-59) and settled in Examples 1 and 3. DNA strands with tengths of 24, 48, or 72 bases (Seq D) and settled in Examples 1.

25 (STACGAGTTGAGAATCCTGAATGCGS' [SEQ ID NO.60]), 4 (STACGAGTTGAGACCGTTAAGACGAGGCAATC-ATGCAATCCTGAATGCG 3'(SEQ ID NO.61]), and 5

(STACGAGTTGAGACCOTTAAGACGAGGCAATCATGCATATATTGGACGCTTT

WO 01/51/65

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unexpectation (6521, 16, 72 m.) were ideal to liked DNAA, A, or G (00, 14, 10 m.). A life precipitation is expectated with a SM CLE, COCKINE, solution to 10 remove excess likely DNA and PNCL.

Lycophilization (10<sup>2</sup> - 10<sup>2</sup> m.) of the aggregate to deprete seasible in pollular stressor of the velotile size, ICE, COCCOMI, Unfortactionalistic, climbs-combilized precision, prompted by the Fern seated, (Fern, Amer. Pero, SM CLI 102-22 (127)) wave dised as a filter and south of comparison purposes. The remaining sixtle significant procedure for the comparison purposes. The remaining sixtle significant procedure for the comparison purposes. The remaining sixtle significant procedure for the comparison purposes. The remaining sixtle significant procedure for the comparison for the sea undust. Significantly, the sixtle DNA-limited angeograpes could be resignified as sixtle procedure. The form of the Comparison for the comparison is the form of the comparison of

The electrical conductoristics of the fines ampites (drief aggregates indeed by 3, 4, and 5, respectively) were measured using a compriser concrolled, from yorker behalinger.

25 Electrical conservation of firms goldwise (2 and 40) are interested submised to prelists with gold potes. Samples were cooled in a moderante vaccuma (10° to 10° tent), and conductivity was measured as the temperature was increased under a day, low pressure or Christian got. The ampite desirative various tentor fluid is node to a

WO 04/51665

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elamines possible optoclessionis offices Barillation, congress were kept in of whole 100 Aux, 4x of the voltage roots of earlies assigned as unimous of 20° X. Supprisingly, the conductivities of the gapaques facune for on all three linkers, require forms of forms of the roots of the roots of the process of the process of the representation of the roots of the roots of the process of the roots of the roots of the process of the roots of the roots of the process of the roots of the r

#### $\sigma = \sigma_0 \exp[-E_s/(kT)] \qquad (1)$

The average activation energies estension from three measurements were  $7.4\pm0.2\,\mathrm{meV}$ ,  $7.5\pm0.3\,\mathrm{meV}$ , and  $7.6\pm0.4\,\mathrm{meV}$  for the 24-, 48-, and 72-met linkers, respectively. Conductivity data from  $50^\circ\mathrm{K}$  to  $150^\circ\mathrm{K}$  were used for these calculations.

Two this disease between the properties of disease types of minerials should depend on the disease between periodics, sparknesses (SASS experiments was used to destinate interposition diseases of the dispensed and sidest aggregates. The SASS experiments was used to the properties of the dispensed and sidest aggregates. The SASS experiments was used to EASS experiments. The Dispense Savet the Dispense Savet the Dispense Savet the Dispense Savet the Savet the Savet the Dispense Savet the Advanced Falson Savet, Aggreen Salieta Liketries, Dispense Savet the Advanced Falson Savet the Savet Sa

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scanning, and sample absorption. The first peak position, which is sentitive a interportion disturber, confession, beauther as where of 0.000 Art. (\*\*0.000mt.) and 0.0371 or "fir the 2.4", 4.9, and Tarser black aggregates, respectively, to an or whose of 0.0371 or "fir the 2.4", 4.9, and Tarser black aggregates conceives. This indicates that is interportive disturbers obseroed ingularity upon dryke, but the point where the precision were demonst tracking, and that intelligent over virtually independent on linder length. This capability why the contract the contraction of the demonstration o

Above 190°C, to measured conductivities of the DNA-fielded simples showed as seconductive display behavior. For all suppose, the conductivity stantal to decrease aboutput a approximately 190°C and ordinated to decrease until approximately 290°C and ordinated to decrease until approximately 200°C. In which point it increased again. To investigate not its session behavior to desirt the electrical conductivity was presented as the image. We as could not warmed expectedly. Therefore(s), the fig. is conductivity only occurred in the direction of images temperature. During DNA is hypotoplate and are conductivity and the conductivity and temperature of the private transcriates, the effect of relative handly used to end that the decident proposition of the hypotoplate content of the conductivity and the supposition of the transcription of the productivity proteoments. From these observations, it was concluded that the transcription of the substitution of the s

PCTYUSBURD 190

measurements on a dried aggregate that was writed with 0.3 M PBS buffer showed a 200% increase in interparticle distance (~2 nm).

These studies are important for the following reasons. First, they show that one can use the molecular recognition proporties of DNA to assemble autoparticle-based 5 materials without passivating them or destroying their discrete structural or electrical properties. If these DNA-functionalized particles are to be used to study electrical transport in three-dimensional macroscopic assemblies or even lithographically patterned structures (Piner et al., Science 283:661-663 (1999)), it is imperative that their electrical transport properties be definented. Second, it shows that over a fairly long linker distance 10 (8 ~ 24 mm), the conductivities of the deied assemblies are virtually independent of DNA linker length. This is likely a result of the removal of water and the use of a volatile salt in these experiments; indeed, the free volume created by removal of solvent and suit allows the DNA to be compressed on the surface and close approach of the particles within the aggregates. Third, the aggregates with the DNA-protected nanoparticles 15 behave as semiconductors, while films formed from citrate-stabilized particles exhibit irreversible particle fusion and metallic behavior. Finally, these results point toward the use of those materials in DNA diagnostic applications where sequence specific briding events between manaparticles functionalized with oligomedicatides and target DNA effect the closing of a circuit and a dramatic increase in conductivity (i.e. from an insulator to a 20 semiconductor) (see next example).

#### Example 22: Detection Of Nucleic Acid Using Gold Electrodes

WO 03/51665

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proquent and standard in 13 mm polar asseptantities as described in Thumphell and 15 mm polar asseptanties a vertice model on the descrice. The color of the glass surface brend pink, infiniting that target 2004, and asseptanties are remarked in 2004 and asseptantial areas assemblies were formed on the glass substantic. Nost, the device was insuranced in 13 Mm. NOLQ, 10 melt produce builder and leasted of 0.0° Sect 10 tools or come respectability to bound 2004, and then treated with a silver statisting solution as described in Discuspio 19 for 5 minutes. The origination of the device leave of 100.

For comparison, a costeni device modified by attacking, oligener/toofce 4, instead of oligener/toofce 1, between the electroctor. Oligener/toofce the the term to copence (\*\*) RESIGNACIONO CANTECACACT (EQ. 100 N.50.39) and oligener/toofce 2 on the annoyatides and will bind to intege DNA 3 on as to prevent binding of the mosquieldes. The test was otherwise performed an described above. The resistance was therefore the 40 NM, the described high of the minister fast was unclearly the control of the second of of the se

This experiment about that only complementary target DNA amound form immegrated unsampled instances there benefitive of the driving and the circuit can be completed by anonymetrical hybridization and subsequent allows standay. Therefore, complementary DNA and recomplementary DNA can be differentiable by missingly conductivity. Bit forms in strateble to durbate strays (shope) with documents of pairs of circuits supplied poly straining for thousands or different models saids another than the straining for thousands of different models saids.

Example 23: Preparation of Oligonschoolde-Medified Gold Nanoparticles using cyclic distrible linkers

In this Example, we character a new cyclic distribution linker for boding collapsemotoridate to gold surfaces, based on steroid distribution 1st (Figure 42) that is simple to prepare, is broadly useful, and affects gold-obligomentorida conjugates coloiditing greater stability soured DTT than those prepared using mercapiolarys linkers. A cyclic distultation was selected as the section of not not become time, our sets deviated as distribution.

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difficused. —5 does were known to form convolutions on a factorized (Neum, et al.), Z.M.
Chem. Soc. 19, 644–445; and a cycle destined worth passably binds to the owners through both rulture carros (Clema, M., MSS 3) billions, June 46-53) to give a feddest structure that cause shifted chanced stables; periodisertners was section at a finding of the contract size in it is restably restable, easily convicated intensiched inch. as a submirror within a time; in production under, undight experior label process or geopenic of visors reached motivation to the gold or derive (Archiver, et al., J. Am. Chem. Soc. 115, 7355-7256 – Biologyapper Clem. 9, 164-640).

- The oligometrolic-good groups used in provious actions were prepared by the execution of oligometrolicities healing numbers recognized program. We good on associationis in an appearsa buffer. They proved to be supprisely brobust, functioning well even after bearings to 100°C certain retirois for 30° years of 5°°C. We have found, however, that these conjugates bear serving in substitution protein serving have made and in substitution containing think, which are by despitations from the contracted objective containing think, which are by despitation parties activated objective found for the good or strate. This future power as profession which has an approprise produce are to be used in a solution containing a final, set for or supply, a FCR hadries that constains distillations of the containing a final set of the containing a final set of the containing a final set of the containing and distillations of the containing a final set of the containing and distillations of the containing a final set of the containing
  - (a) General
- NAME specim were recorded on 500 NoTic ('H) and 400 Molte ("P); exquisition at 161 Molte; Virigina spectromates using CDCs; as a solvent set TLMS as an internal (1) and 400 Molte; Virigina spectromates using CDCs; as a solvent set TLMS as an internal (1) and 400 Molte; which is solven to the solvent of th
  - (b) Preparation of Steroid-Disulfide Ketal (1s)
  - The synthetic adverse is shown in Figure 43. A solution of epismdrosterone (0.5g), 1,2-dibbiane-4,5-diol (0.28 g), and p-tolurenesulfonio acid (15 mg) in tolurene (30 ml), wate refluxed for 7 in under conditions for removal of water (Does Stark apparatus).

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PCT/USBL/II 150

- 10. Cognosticologica State (Contention State (Co
- 20 (d) <u>Preparation of 5'-Modified Olimponuticolides and Nanopartials</u>

  Conjugates
- 3"Modified algorotomical is it and Lick were constructed on CPG support using conventional hyphophandistic Assertion, except that composed in Pligras 43 lives complexed in the final phosphilatinian step. Products were cleared from the support by sensors with concentrated NRAGE for the fin 45 °C". The cligamotomical was regarded by prevention plant PEGF or a Discour. DRAGE system colored for NRAGE systems. DRAGE special value for the plant PEGF or a Discour. June present in stay using TRAG Moffer (BET 30) and \$10 ftmm; partices of SPS CEGFS MOD STACK AS A Serve set of 1.

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mblanic. Antion frames of the shopsybolect cannot group in that the reprod derivatives requirest cheality for unsupposition group. The control of the contr

It inch of the modified disputationides was investibilized on ~12 ms gold magazinites by presentes and the materia plainus electric strength a memorphismy) hand group (Storberff et al. (1999) J. Am. Chen., Son. 120. 1995-1946.). This investived auditing thirst attributed managarithists (~12 ms in dismertal) for \$1 house in a bottler and indicate causining an observationide bearing a travelula in \$10 feet and \$10 feet in \$10 feet i

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To evitatus the utility of management endes produce countriesing the
distributions and notice was present graches (cf., icf., icf.,

WO 01/51665

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nanoparticle. Hybridization of pairs of nanoparticle probes with target oligonucloolides leads to formation of fixee dimensional narworks and a classage in color from red to blue-gray (Mocic, R: C., et al., J. Arn. Clera: Soc. 120, 12674-12675).

Hybridization of the probes was examined using a 79-mer oligonucleotide 5 targets, containing sequences complementary to the probes (Figure 43). The reactions were carried out at room temperature by adding 1  $\mu L$  of the target solution (10 penol of IV) to colloidal polutions of the probe pairs let, Je2, and Het and He2, and He1 and He2 (50 µL and 1.0 A<sub>80</sub> Unit of each manoparticle probe) in 0.5 M NaCl, 10 mM phosphore (pH 7.0). At times 10 seconds, 5 minutes, and 10 minutes, aliquots (3 µL) were removed 10 and spotted on a C-18 reversed phase TLC plate. The various probe pairs all behaved the same: the spots for the 10 second reactions were red, indicative of free nanoparticles; those for the 10 minute reactions were deep blue-gray, characteristic of aggregates of manaparticles; and the 5 minute reactions offerded spots with a reddish blue color, indicative of a mixture of non-associated and associated nanoparticles. In agreement 15 with previous observations for aggregation of nanoparticles effected by hybridization of oligonuclassides (Stochoff, J. J., et al., J. Am. Chort. Soc. 120, 1959-1964; Elghanian, et al., Science, 277, 1078-1081; Maxie, R. C., et al., J. Am. Chem. Soc. 120, 12674-12675; Mitchell, G. P., J. Am. Chem. Soc. 121, 8122-8123), the reactions were reversible. Thus, warming the aggregate mixture to 90 °C (above the dissociation temperature for the 20 oligorates linking the nanoparticles together) and spotting while hot afforded a red spot. Por control exportments in which the oligonacteotide target was conified or was not

We conclude that the namoparticle conjugates guinested via the namod-familife such care function of floritory in sphediatesten proches. Moreover, as judged by the upon test, 25 the conjugates with the various another substances with the super object-scholdest at convenienth trans. This floriest is consistent with expectations for probe having comparable transit set of oligometroleodess on the surface of the namoparables consistent set of oligometroleodess can be surface of the namoparables and machine the complicion regions relatively that removed from the 5°-bast groups.

complementary to the probes, the color was red under all conditions.

WO 01/51615

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Reaction of Nancounticle Probes with Dishiothesical.

Addition of thick to collected solutions of gold nanoparticles or gold

Addition of little to collected someters or gots nanoparticles on gots sameparticles loaded with mercaptohexyd-obigonuclocities leads to aggregation of the manoparticles. The color changes from red to deep blust, and on standing a dark

- 5 procipilata settles cut. An demonstrand with experientes with assopratials boaring Douesexezity lateled oligonorleotides, the third displaces the mercapholityloligonocleotides bound at the gold surface (Motic), R. C., (1999) Synthesis Programmable Nanoparities Assembly Using DNA, PBD Thasis, Northwestern Labracia? In noceatast to assembly Using DNA, PBD Thasis, Northwestern Labracia? In noceatast to assembly in induced by Indiagence of oligonate-totidetides.
- Programmine Nanophetto Assentory Using U.N., 1201 Instan, Numerocam University). In contrast to aggregation induced by hybridization of oligometeoridenenoparticle conjugates, three rescrivoss are irroversible, neither healing nor addition of NaOM disassembles the aggregates.
- We have used this color to monitor the receition of DTT with probes prepared with the secreted cyclic similation, the mercupathneys, but the suprilet classifies being appears. The experiments were carried and by adding a 1 µ. of 1 th DTT in watering to 100 µ. of 1 to 15 moneyartic-cologonactivaties probe audition (2 Augus bilas commercially) in 6.5 M NoCl and 10 mM phosphate (pdf 7.0), then specifies 3 µ. is deployed on a TTC-plane at various and 10 mM phosphate (pdf 7.0), then specifies 3 µ. is deployed on a TTC-plane at various
- times not observing the colors. As aboven in Table 1, reliability produce derived from obspeculationities with the mescapethorty (Red and Rel) and acyclic object-model headurgroup (III ca) incided regiolly. A reliability port was obtained to 30 seconds and a 20 steeps (bit up or within 5 minusus. By 100 minusus, most of the gold had precipitated. In contests, so color change was observed for the restrict on 0 the product prepared with the method coveril designable head group (CHL 20) within 60 minuses. It had, 160 minuses to the color of the color observed for the restrict on 0 the product prepared with the method coveril designable head group (CHL 20) within 60 minuses.
- reach this scene order administed with problem programed with Eleh, List, or with Birls in 20 seconds. On this beats, we estimate that the react of resolves of this second distulted 20 problem with DTT is of the order of LOMOP that of the order problem. The problem program from the scyclic distultible another is reached as the following proposed from the scyclic distultible another is reached as about the same rate as the problem programed with the anteroptic bearing size for. The latter result is not supplishing in view of orderone that the section of an anyolic distultible with good probably involves change of the 5.5 in

WO 03/51/45

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band (Zhong, C. J., Leagunir, 15, 518-525). Accordingly, an oligonucleatide with an acyclic head group would likely be linked to gold through a single sulfur atom, so in the case of mecropolacyl-oligonucleotide derivatives

To see if grobes present of from let not it in the till now an hybridization probast after installe just dependent profession og to relative for original originalise for the probase with DVT index the conditions on the first from a finish in Table I. After 20 militars, i, jul of a solution of the 79 meet regard eigenstation (of 0, 1900) was added to one. Both supplies were then employed indexed to their, and analyse by the specific conditions of the 70 meet regard to their, and analyse by the specific test. The specific form of the employed control in the profession of the control in the profession of the specific configuration of the object seeds of the specific configuration of the object seeds of the specific configuration of the specific configuration of the specific configuration of probles desired from the memorphological district from th

Table 1. Colors from reactions of Gold Manaparticle Probes with DTT

1c1 + 1c2	Time 0	20 sec	S min	40 min	100 min
	red	red	zed	red	red-bluc
tiei + 11e2	red	red-blue	blue	blue	(black prec.)
Hiel	red	red-blue	blus	blue	(black prec.)

#### (g) Constusion

"Guida narropericle eligenmeleoside conjugates moles using this cyclic distallide timbre serve as effective probes for destelogs speciale oligenmeleosides sequences, and day subhili mode generate shallijs sowned distribution liban compropriating engingetes propered with the conventional merceptolecy group or as asystic distallides with. The high shallight process filed destribution libary treating, in part is sets, from archeologic exWOODSHIE

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eligenspicestide to sold through two sulfur atoms.

Example 24: Pregaration of Oligomechonide-Modified Gold Nanoparticles using a simple cyclic disalfide linker.

In this Example, we prepared a non-steroid cyclic disulfide linker and oligonuclectride-nasoparticle probes from this linker and evaluated the probes stability in the presence of thiol-containing solutions relative to probes prepared with steroidal cyclic dissatifide and alkyl thiol linkers. Procedures have been described for preparing probes for detecting DNA or RNA sequences by binding objects confide to gold manaparticles 10 using slkylthiol anchor groups, 1, Figure 44 [C. A. Mirkin et al., Nature, 382, 607 (1996); Storthoff, et al. J. Am. Chem. Soc., 120, 1959 (1998)] or a steroid cyclic disulfide anchor group, II. Figure 44 (R. L. Letsinger et al., Bioconjugate Chemistry, 11, 289 (2000)]. As probes, the conjugates prepared using the steered cyclic disulfide linker have proved advantageous in that they are much more stable in the presence of thiol compounds, such 15 as merceptoethanol or dithiothreitol (DTT), then are conjugates prepared using an alkyldriol anchor. This feature is important since PCR solutions employed in amplifying DNA samples for detection contain small amounts of DTT to protect the enzyme. For simple and rapid detection of PCR products it is desirable to use probes with high mebility toward DTT so that the test can be cerried out directly in the PCR solution 20 without having to first isolate the amplified DNA.

The finance desimpation that stead option makes (composed. It Figure 42()). The cyclic distribute, which can be principle provide two binding often also conducted to the conduction of the cond

Compound 2a, prepared by heating trans-1,2-diffusno-4,5-dithiol with acred in

PCT/US#I/01390

tolurne, was converted to a cyanosthyl N,N-di-i-propyl phosphoromidite reagent, 2b, which was employed in the final coupling step in the synthesis of modified oligensecteotides 2c) and 2c2. One gold conjugate prohe was prepared by treating a gold colloid solution with 2c1 and an equimoter amount of 2d, which serves a diluced on the 5 gold surface. A comparion probe was made from 2c2 and 2d in the same way. These nanoparticle conjugates were stable in a range of solutions of sodium chloride (0.1, 0.3,

### 0.5, 0.7 M), both en stending and on freezing and thawing. (a) Preparation of compounds 2s. 26, 2c1 and 2c2.

Compounds 2a was prepared as described in Example 23. Phosphisilation of 2a 10 and synthesis of of oligonucleotides 261 and 262 were carried out as described previously for the steered cyclic disulfide derivatives in Example 23 and elsewhere [R. L. Letsinger et al., Bioconjugate Chemistry, 11, 289 (2000), the disclosure which is incorporated by reference in its entirety). The time of reaction in the step involving condensation of 18th with the oligomer on the CPG support was 10 min.

(b) Preparation of Gold-Oligomusleutide Conjugates. Equimolar amount of oligonacleotides 201 and 2d or 2c2 and 2d were added to 13 am sold colloids (~ 10 nM) to provide solutions containing 1.7 amole/mL of each oligosuclootide. The solutions were stored in the dark for 24 h; then salts were added to make the solutions 0.3 M in NaCl, 10 mM in phosphate (pH 7.0), and 0.01% in sodium 20 sgide. After 24 h the NaCl concentration was increased to 0.8 M and the solution were allowed to stand for another 24 b. The colloid was then filtered to remove any aggregates and the solution was centrifuged to collect the nanoparticles. The pellets were washed with nanopure water, recentrifuged and redispersed in 0.1 M NeCl, 10 mM phosphate buffer (pH 7.0), 0.01 % sodium azide.

(c) Reaction of nanoparticles proles with dithiotherital Displacement studies were carried out at room temperature (22°C) by adding 2  $\mu L$ 

of 0.1 M DTT to 20 µL of a mixture of equal volumes of the collectal conjugates obtained from 2c1 and 2c2. Aliquots (3 µL) were periodically removed and spotted onto WO GAS 1665

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a white Nylon membrane. Initially the spots were not Displacement of the obligacement of the obligacement of the part of the p

- The competition, disposalcoded conjugate were infinitely prepaid from objections of the conjugate and the conjugate and
- 20 Example 25 Erganglon of Dissounted tick-Modified folds Monostricias to this Example, we evaluate the stability of a new anythic disable inkers, a tribiol, relative to daily fall and meterodal cyclic disables inkers in the presence of folioc centricing arbitraries. For compression, oliganucleotidas with a morcupathoxyl another (exemption). Figure 43) and with a sterooid cyclic disables making feorogrand 6, Figure 25.
  25 45) were present.
  - (a) Symbosis and characterization of 5'-tri-meraposalized elisponucleotide. The 5'-trimerespitoelizythiol oligonucleotide was symbosized as shown in Figure 47. Trembler phosphoramidite 7 (Glen Repearch Inc. Sterling, VA), Figure 45, and Thiol-

WO 91/51/45

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modifier CS S is jumplementaled, www revenuity required to the S' and of a protocol adjacent at all broads in the CPG support. The protest was classed from the CPG and profiled as shortly above. The restated term far reliable objects was classed from the CPG and profiled as shortly above. The restated term far reliable objects are shortly arranged by the classed of the Diff. From one to the production of the classes of the Diff. From one to the production of the classes of the Diff. The contraction of the composers was to add the composers. The objects was related was related to the Diff. The restated from the Diff. The restated train as fails in State of the CPG and th

(b) <u>Provision of 2-30 alor of ministria Provision from Section 2018</u>, assessable, Gold anaporticles were used a paradas from Vortec 1-40 contraction (Undergame, Co.) 7 o 10 cd., of 70 mp poll evides of most decided on a decided of 20 cd attain modelled DNA. The solution who whought to 10 XM recipion of word purphysis harding of 10 xH. and poll provision of 20 cd 20 poll provision. Then the assessable to we show of the other hospitals who who who contributions are solved in the contribution of 20 cd 20 poll provision. The collection is upwarrant, the notice provision are solved as to the contribution of 20 poll provision. The collection is poll provision are an explanated in 20 poll provision. The collection was weaked twice more using 10 cd fisch FISS buffer by requesting that provision are solved to the contribution of 20 poll provision.

(c) Skablick test of fisied DNA modified and measuraists Solid DTV us added to 8000. 4 actions of the different prior of fisiel- or desided DNA modified 30 am gold amosparticle colloids with the DTT concentration was 0.017M. As DTT displace the diagnostication, for bother of the colloid terms from each of bate UVVVIS openits were taken as a function of lime. The attendances of a state of the bate of the displacement of the colloid terms from

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americant with disposal Dions gold particles began in decrease and a bound hand at 1970mm began is grow. The born of 700 me is associated with collecting aggregation, As above in Figure 15, Juny 1 and old edigenometrated (c) 1 and fill of 20 me gold particles quickly from an aggregate in 0.077 by 1771, 2017 is 11.3 bound, the collection beautify sense below 1770 by 1771, 2017 is 11.3 bound to considerable towns block and the collection beautify and the degree of the collection because the collection

All patents, patent applications and references chad herein are bereby incorporated by reference in their entirety.

WO 01/51/65

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WECLAIM

- A method of detecting a nucleic acid having at least two postions
- providing a type of nanoparticles having oligonacleotides anothed thereto, the 
  5 oligonacleotides on each meaparticle having a sequence complementary so the sequence
  - of at least two portions of the nucleic sold, contacting the nucleic sold and the nanoparticles under conditions effective to allow hybridization of the oligonucleotides on the nanoparticles with the two or more
- portions of the nucleic sold; and

  observing a detectable change brought shout by hybridization of the
  oligonous cotides on the nanoparticles with the nucleic sold.
  - A method of detecting nucleic and having at least two portions comprising:
- 15 consisting the models and what is test two types of emoperhelia bening adigenociroliset annial dresses, the dispensionless on the first period of assequential testing a superson complementary to a first portion of the requirate of the medical soil, the officinationalises on the encount type of assequentiates traving a sequence complementary to a second period on the exceptor of the credit soil, the contributing the period of the credit soil, and the contributing that the period of the credit soil and the medical soil, and the temple soil and the period of the obligation of th
- observing a detectable change brought about by hybridization of the oligonucleotides on the nanoparticles with the nucleic acid.
- The method of Claim 2 wherein the contacting conditions include freezing
  - 4. The method of Claim 2 wherein the contacting conditions include hearing.

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- 5. The method of Claims 2 wherein the detectable change is observed on  $\boldsymbol{u}$  solid steffice.
- The method of Ctaim 2 wherein the detectable change is a color change observable with the naked eye.
  - The method of Claim 6 wherein the color change is observed on a solid
  - The method of Claim 2 wherein the nanoparticles are made of gold.
- 9. The method of Claim 2 wherein the oligonucleosides attached to the manoparticles are labeled on their ends and attached to the nanoparticles with molecules that produce a delectable change upon hybridization of the oligonucleosides on the nanoparticles with the nucleic axid.
- The method of Claim 9 wherein the nunoparticles are metallic or semiconductor necessaristies and the oligonucleotides attached to the nanoparticles are blocked with filterescent molecules.
  - 11. The method of Claim 2 wherein:
- the nucleic acid has a third pertion located between the first and second posticess, and the sequences of the oligenucleotides on the natesparticles do not include 25 sequences complementary to this third portion of the nucleic acid; and
  - the nucleic acid is further contacted with a filler oligomolecticle having a sequence complementary to this third portion of the nucleic sold, the contacting taking place under conditions effective to allow hybridization of the fifter oligonacleotide with

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## the nucleic acid.

- 12. The method of Claim 2 wherein the nucleic acid is viral RNA or DNA.
- 13. The method of Claim 2 wherein the moleic sold is a gene associated with
  - 14. The method of Claim 2 wherein the nucleic sold is a bacterial DNA.
    - 15. The method of Claim 2 wherein the nucleic acid is a fungal DNA.
- 16. The method of Claim 2 wherein the nucleic socid is a synthetic DNA, a synthetic RNA, a structurally-modified outural or synthetic RNA, or a structurally-modified natural or synthetic DNA.
- The method of Claim 2 whorein the nucleic solid is from a biological
  - The method of Claims 2 wheecin the medicin acid is a product of a polymerase chain reaction amplification.
  - The method of Claim 2 wherein the nucleic acid is contacted with the first and second types of meroparticles simultaneously.
- 20. The method of Claim 2 wherein the multic acid is contacted and 25 hybridized with the oligonuclootides on the first type of nanoparticles before being contacted with the second type of nanoparticles.
  - 21. The method of Claim 20 wherein the first type of nanoparticles is suached

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#### to a substrate.

- 22 The method of Chim 2 wherein the nucleic acid is double-stranded and hybridization with the oligonucleotides on the nunoparticles results in the production of a triple-stranded correlex.
- 23. A method of detecting nucleic acid having at least two portions
- providing a submrate having a first typo of nanoparticles attached thereto,

  10 the nanoparticles having oligonucleosides attached thereto, the oligonucleosides having a
  sequence complementary to a first portion of the sequence of a metaloic and to be
- consecting said mustels acid with the nanoparticles attached to the substante under conditions effective to allow hybridization of the oligonacleotides on the 15 nanoparticles with said nucleic scid;
  - providing a second type of nunoparticles having oligonucleoides attached thereto, the oligonucleoides having a sequence complementary to one or more other portions of the sequence of said moleic soid;
- connecting said nurthele acid bound to the substates with the second type of
  manopasticles under conditions effective so allow hybridization of the oligonuclivoides on
  the second type of manopasticles with said sectics acid; and
  observing a detectable chance.
- 24. The method of Claim 23 wherein the substrate has a plurality of types of nacoparticles attached to it in an array to allow for the detection of multiple portions of a single nucleic acid, the detection of multiple different recicle acids, or both.
  - 25. A method of detecting nucleic acid having at least two portions

PCT/ESUL/01390

comprising:

providing a substrate having a first type of recopericles attached theseto,
the mesoparticles having oligonacleosides attached thereto, the oligonacleosides having a
sequence complementary to a first portion of the sequence of a metric acid to be
detected:

- contacting said mucleic acid with the renoparticles attached to the substante under conditions effective to allow hybridization of the oligonachoolides on the nanoparticles with said mucleic soid;
- providing a second type of asnoparticles having oligonucleotides attached
  thereto, the oligonucleotides having a sequence complementary to one or more other
  portions of the sequence of said modele ocid;
  - constating said nucleic axid bound to the substrate with the second type of manaparticles under conditions effective to allow hybridization of the oligenucleotides on
- the second type of nanoparticles with said nucleic soid;

  15 providing a binding objecture with said nucleic soid;

  less two portions, the first portion being complementary to at least a portion of the
- least two portions, the first portion being complementary to at least a portion of the sequence of the oligonacteorides on the second type of nanoparticles; contacting the binding oligonacteoride with the second type of
- nanoparticles bound to the substrate switer conditions officially to allow hybridization of
  the binding oligonactoritie to the officenteeroids on the manoparticles;
  - growiding a third type of nanoparticles having oligonucleotides attached thereto, the oligonucleotides having a sequence complementary to the sequence of a second portion of the binding oligonucleotide;
- contacing the third type of manoparticles with the binding oligonucleotide

  55 beand to the ministrate under conditions effective to silve hybridization of the binding

  oligonucleotide to the oligonucleotides on the nanoparticlet; and

observing a detectable change.

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26. The method of Cheim 25 wherein the substrate has a plurality of types of annoparticles estached to it in an array to allow for the detection of multiple portions of a single nucleic acid, the detection of multiple different models acid, or hoth.

- A method of detecting nucleic acid having at least two portions comprising:
- contesting a muscles social to be detected with a substates having oligonationides standard distrete, the oligonationides having a sequence complementary to a first portion of the sequence of said assetics social, the contenting taking place under 10 conditions effective to allow hybridization of the oligonationsdar on the substates with said miscled social.
- constanting and practice and bound to the substants with a filest type of amountaining the survivage over one types of dispersociotides statisted themsels, at least one of the types of dispersociotides the substanted themsels, at least one of the types of dispersociotides the substantial as respective compromensatory to a second protect of 5 the segentes of said motion said, the conducting taking place unfor conditions effective to allow hyportation of the enliquentiation of the motion said.
- containing the first type of managementals bound to the abstrates with a second type of managementals baving originatelectrics stated themes, the object-missionist can the second type of managemental baving a requires complementality 20 to a last a portion of the sequence of one of the types of objectimationise on the first type of amountains, the containing tealing place unfort contains of districts to 2000 hybridization of the objectimations on the first not second types of managements, and districts and the containing teal to the containing the c
- 28. The method of Chima 27 wherein the first type of nanopartisles has only one type of oligomutionides statched thereto, the oligomutionides having a sequence complementary to the second poeting of the sequences of said trachies and and to at least a precision of the sequence of the oligomatelentides on the second type of passoparticles.

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- 29. The method of Claim 28 further comprising contacting the second type of campourities bound to the oblatice with the first type of campounities, the contacting taking place under conditions effective to allow hybridization of the oligonateleoides on the first and second types of sanoparticles.
- 30. The method of Chine 29 wherein the first type of numperiaties has at least two types of oligometeristics attacked insectes, the first type of oligometeristics having a sequence complementary to the second prime of the sequence of said medicine and, and to the accord type of oligometeristics having a sequence complementary to the sequence of at test as position of the oligometeristics on the second type of consperticular.
- 31. The mothed of Chies 30 faulter comprising contacting the second type of sunsparticles bound to the reletate with the first type of susparticles, the controling 1s taking place under conditions effective to allow hybridization of the oligonacteolides on the first and second types of numperateles.
- 32. The method of Chim. 27 wherein the autotrate has a pleasity of types of oligonacleosides attached to it in an erray to allow for the detection of multiple portions of a single medicia acid, the detection of multiple different nucleic acids, or both.
  - 33. The method of any one of Claims 23-32 wherein the substrate is a transparent substrate or an opaque white substrate.
  - 34. The method of Claim 33 wherein the detectable change is the formation of dark areas on the substrate.
    - 35. The method of any one of Claims 23-32 wherein the panoparticles are

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made of gold.

- 36. The method of any one of Claims 23-32 wherein the substrate is controlled with alliver stain to produce the detectable change.
- The method of any one of Chines 23-32 wherein the detectable change is observed with an optical scenner.
- A method of detecting nucleic solid having at least two portions
   comprising:

contacting a motific sold to be directed with a substress having objective that the directes, the objective having a sequence consistenctivy to a first portion of the sequence of said motific sold, the contacting billing place coder motifies effective to allow hybridisation of the objective on the substrate with said motifies on the substrate with

contesting said mediate and bound to the substrate with a type of campuraticles having disputated state attacked reterent, the disputated state at sequence complementary to a second position of the sequence of rada wateria said, the contenting taking place under conditions effective to allow hybridization of the conjugated continues on the nanoparticles with said structice continues.

contacting the substrate with silver stain to produce a detectable change;

observing the detectable change.

- 39. The method of Claim 38 wherein the manoparticles are made of a noble costal.
  - 40. The method of Claim 39 wherein the nanoparticles are made of gold or silver.

WO 01/51/65

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- 41. The method of Claim 38 wherein the substrate has a plurality of types of oligonucleotides attached to if in an array to allow for the desection of multiple portions of a simple nucleic acid, the detection of multiple different nucleic acids, or both.
- The method of any one of Claims 38-41 wherein the detoctable change is observed with an optical scanner.
  - 43. A method of detecting aucleic solid having at least two portions
- contasting a muchoic solid to be detected with a substante having oligomucleosides attacked thereto, the oligomucleosides abving a sequence complementary to a first portion of the sequence of sub localicie solid, his contasting taking place under conditions effective to allow hybridization of the oligomucleosides on the substants with said muchoic solid.
- 15 containing said notation said bound not the substrate with ligonomers having oligonomecolotical submide thereon, the alignosectoridate having a sequence complementary to a portion of the sequence of axial medica said, the contacting taking place under conditions of first extra of axial medica said, the contacting taking place under conditions of first vity to allow hybridisation of the alignosectorides on the lipseomers with said posterior said.
- 20 containing the lipoteness bound in the schemes with a few type of mesograticies having at least effect type of signaturclocified standard factors, the first type of originate/confect standard factors, the first type of originate/confect incurs a phylophobile grows intelled to the cnd and statuable to the managearielise, the contenting taking place under conditions effective to allow standarders and an experience of the transportation of the properties of
- observing a detectable change.
  - 44. A method of detecting nucleic sold having at least two portions

WO 03/51/45

PCT/USBIADI 150

comprising

contacting a muscle said to be detected with a substrate huring oligomenterities attached threets, the eligomenterities having a sequence complementary to a first portion of the sequence of said matrix said, after contacting taking place under 5 conditions effective to allow hybridisation of the oligomenterities on the substrate with said studies only.

contacting sale medic aid bound on the substrace with Speponess having oil gazum-incides statuched thereon, the eligenucleotides having a cojumne complementary to a portion of the sequence of said audien aid, the contenting taking place under to except the contention of the sequence of said audien aid, the contenting taking place under to except the said audient aid of the cili generated and the said procedure on the Specomes with said numbries said.

contending the liposomes bound to the substrate with a first type of newsparticles having at least find type oligonucleotidate attached themses, the first type of oligonucleotidates having a hylophobolic group stratebal on the and not attached to the substrated having a hylophobolic group stratebal on the and not attached to the 1st amountained to the contracting tables place under conditions efficient to allow statements of the oligonucleotidas on the subsyriations to the liposomes as a result of hydrophobic disc.

contacting the first type of nanoparticles bound to the liposomes with a second type of nanoparticles having oligonucleotides aluehod thereto,

b) fine first type of nanoparticles having a second type of oligomacicotides attached thereto which have a sequence complementary to at least a portion of the sequence of the oligomosteotides on the second type of nanoparticles,

the oligonateleatides on the second type of manoparticles having a sugressor complementary to at least a portion of the sequence of the second type of oligonaricotides on the first type of manoparticles,

the contesting taking place under conditions effortive to allow hybridization of the oligonucleoides on the first and second types of nanoparticles; and observing a detectable change.

WO 03/51655

PCT/USBL/B1190

- 45. The method of Cisina 43 or 44 wherein the substrate has a planshity of types of oligonusteotides attached to it in an array to allow for the detection of multiple portions of a single naticia sold, the detection of multiple different musicia solds, or both.
  - 46. The method of Claim 43 or 44 wherein the nanopurticles are made of gold.
- 47. The method of Claim 43 or 44 wherein the substrate is contacted with silver stain to produce the detectable change.
- The method of any one of Chims 43 or 44 whereio the detectable change is observed with an optical scanner.
- 49 A method of detecting multile still having at least two partices 15 comprising:
  - providing a substrate having a first type of nanoparticles attached threeto, the manoparticles having oligonocleosides attached threeto, the oligonocleosides having a sequence complementary to a first portion of the sequence of a modelo acid to be
  - contacting said machine solid with the nincoparticles attached to the authorized under conditions effective to allow hybridization of the oligonacteolides on the managemiotes with said outled acid;
- monopartities having all aggregates probe comprising at least two types of monopartities having oligonoclassidae autocide directs, the enceparities of the aggregate 25 probe being located used to these a create of the hybridation of some of the oligonoclassidaes attached to those, at least one of the types of samoquities of the aggregate probe having oligonoclassidaes attached thereto which have a sequence consultant properties of the aggregate probe having oligonoclassidaes attached thereto which have a sequence consultant properties and the composition of the properties and the composition of the properties of the composition of the properties and the composition of t

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performance and

contacting said nucleic soid bound to the substrate with the aggregate probe under conditions effective to allow hybridization of the oligonaucheolides on the aggregate probe with said models acid; and

observing a detectable change.

50. The method of Claim 49 wherein the substante has a plurility of types of nasoparticles attached to it in an array to allow for the detection of multiple portions of a single multito sold, the detection of multiple different nucleic sold, or both.

 S1. A method of detecting smelcic acid having at least two portions comprising:

providing a rubstrate having oligonucleotides attached thereto, the oligonucleotides having a sequence complementary to a first portion of the sequence of a sucleic acid to be detected;

15 providing an aggregate probe comprising at least two types of emorphistics having collegenderates attached thereto, the nanopartities of the aggregate probe being loads to each other as result of the hybridism of some of the oligencelevities satested to them, at least one of the types of imagentaties of the aggregate probe having oligenselecticies annualed thereto twich have a resource complementary as except profice of the separan of and stuckies said.

contacting and austeric acid, the substrate and the aggregate probe under conditions effective to allow hybridization of raid auxidio acid with the oligonucleotides on the aggregate probe and with the oligonucleotides on the substrate; and observing a detectable change.

52. The method of Chriss 51 wherein said models acid is constanted with the substrate so that said nucleic acid hybridizes with the ollgonucleotides on the substrate, and said models until bound to the substrate is then contacted with the aggregate probe so

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that said nucleic acid hybridizes with the oligonucleotides on the aggregate probe.

- 53. The method of Claim 51 wherein said motive soil is contented with the aggregate probe so dat said motives early hypordises with the oligomethodistics on the 5 aggregate probe, and said motives and bound to the aggregate probe is then conducted with the substrate so that said materia said hybridizes with the oligomethodistics on the conductive of the conductive said the oligomethodistics.
- 54. The method of Claim 51 wherein said nucleic acid is contacted to significanceusly with the aggregate probe and the substrate.
  - 55. The method of Claim 51 wherein the substrate has a plurality of types of oligonucleopides attached to it in an array to allow for the decedion of multiple portious of a single multic acid, the detection of multiple different nucleic acids, or both.
  - 56. A method of detecting washers acid baving at least two positions comprising:
- providing a substanta having olligenschoolides statisfed fluority, providing as aggregate probe competing at last the types of 20 monopartism it state to types of 20 monopartism its state olligenschoolides anniholid dorson, the monopartism are gregority to cost o their as a result of the hybridisation of some of the olligenschoolides are their as a result of the hybridisation of some of the olligenschoolides are their and the state of the types of impossible of the aggregate youth lasting olligenschoolides attended destro-which have a sequence complementary to fair profession of a resultion and the destroich.
- providing a type of manoparticles having at least two types of oligometeodists stunded thereto, the first types of oligometeodists baving a sequence complementary to a second position of the sequence of said studeled said, the secondport oligometeodide lawing a sequence complementary to at least a portion of the

WO \$1/51665

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sequence of the oligonucleotides attached to the substrate;

controling tall matche said, the agenyate prob, the assuperations and the salarants, the contesting taking place under conditions affective to allow hybridization of said arcticle and with the observations on the aggregate peaks and on the 5 assuppractices and hybridization of the edigenous/ordines on the numerous contesting the conte

observing a detectable change.

- 37. The method of Chaim 56 whereis said totalex axis is consisted with the aggregate probe and the assoparticles so that and suchsis acid typicidates with the edigenoushcation on the aggregate pobe and with the ollowardsoldes on the managementicles, and said nucleil axid bound to the aggregate purbor and assoquaticles in these connected with the relations to that the ollowardsolders on the managementicles highly darked with the objective closely only of the production of the managementicles highly darked with the objective closely only of the production of the managementicles.
- 33. The included of Chains So wherein said mobile sold is constanted with the aggregate probe 10 that said models said by hydralizer with the eligenmentations on the aggregate probe, and models used bound by the aggregate probe in the constanted with the models of the said production of the said production of the said production of the said production of the consputed on the said conducts and bound to the aggregate probe and assemption in the constanted with the substante so that the eligenmentation are assemption in the constanted with the substante so that the eligenmentation are the sanopsimiles.
- 39 The motiled of Claims Se wherein sold models said is constanted with the aggregate probe on that said models and hybolition with the oligomethodistics on the aggregate probe, the management can be constanted with the subdends so that the oligomethodistics on the management of the said constant of the oligomethodistics on the management of the subdends with the oligomethodistic or the subdends with one of the value of the five constanted with the subdends was the value further on the real presidence in time constanted with the subdends with one of the subdends of the subdends on the subdends of the subdends of

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nanoparticles bound to the substrate so that said mucleic sold hybridizes with the oligonacteotides on the nanoparticles.

- 60. The method of Claim 56 wherein the substrate has the oligonucleotides attached to it in an array to allow for the detection of multiple pertions of a single nucleic acid, the detection of multiple different nucleic solds, or both.
  - The method of any one of Cloims 49-60 wherein the substrate is a transported substrate or as openque white substrate.
  - 62. The method of Chaim 61 wherein the detectable change is the formation of dark areas on the substance.
- 63. The method of any one of Claims 49-60 wherein the nanoparticles in the 15 aggregate probe are made of gold.
  - 64. The method of any one of Chains 49-60 wherein the substaste is contacted with a silver stain to produce the detectable change.
- 65. The method of any one of Claims 49-60 wherein the detectable change is observed with an optical scanner.
  - 66. A method of detecting muoleic acid having at least two portions comprising
  - contesting a nucleic acid to be detected with a substrate having objects attended to the contesting a requires complementary to a first person of the requires of said succisio said, the contacting taking place under conditions effective to allow hybeidistation of the objects object with the adultative with

WO 01/5.065

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said notleic said;

constating said wateria said bound to the substries with inpromose having obligated codes attached florests, the oil genuelectrists having a sequence complementary to a portion of the requirect of a said matchine acid, the contenting labeling place under 5 conditions affective to oflow hybridization of the oil genuelectrides on the liposomes with said exactles.

providing an aggregate probe comprising at least two types of menoproficies having ollipsociated annihold throne, the nanoproficies having ollipsociated annihold throne, the nanoproficies of the aggregate probe holips (board to each other as result of the hybridization of some of the digeometrosides attached to them, at heat one of the types of annoparticles of the aggregate probe having olipsocietoddes attached throne which have a hydrophobic group statched to the end not attached to the annoparticles.

contacting the Esponemes bound to the substrate with the aggregate probe under conditions effective to allow attachment of the oligonuclostides on the aggregate 15 probe to the liposonucs as a result of hydrophobic interactions; and

# observing a detectable change.

 The method of Claim 66 wherein the nanoparticles in the aggregate probe are made of gold.

68. The method of Claim 66 wherein the substrate is connected with a silver stain to produce the detectable thange.

69. The method of Claim 66 wherein the substrate has a plurality of types of 25 oligonucleoides attached to it in an array to allow for the detection of multiple postions of a niggle medicie and, the detection of multiple different outside acids, or both.

70. A method of descenting nucleic acid having at least two portions

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# comprising

- providing a substrate having oligonucleotides attached thereto, the oligonucleotides having a sequence complementary to a first portion of the sequence of a muticle acid to be detected;
- providing a one pube competing us loss two types of manaparticles, each type of manaparticles having oligomeoleosides stim-his throots which are competentary to the oligomeoleosides on at least one of the other types of temperaticle, the excepticities of the aggregate probe being bound to each other as a vesuit of the hybridistation of the oligomeoleotide antabods to licen;
- providing a type of manoparticles having two types of oligomecleosides standard faceto, the first type of oligomecleotides having a sequence complementary to a second portion of the sequence of salar nucleia sadd, the second type of oligomecleotides lawing a respector complementary on a position of the sequence of the eligomecleotides tambed to a last ease of the types of imagentatics of the core poles;
- contacting and nucleic acid, the anaparations, the substate and the core
  probe under conditions effective to allow hybridization of said nucleic acid with the
  oligamentonicies on the ranoparticles and with the oligamentonicies on the substate and
  to allow hybridization of the oligamentonicies on the substate with the
  oligamentonicies on the core probes, and

# 20 observing a detectable change.

7. The perhaps of Chilm 70 rhemin and motifies shall be constanted with the systemate so that said ancide axed hypoditers with the originated motifier on the withtrain, and said motifier dead bound to the subheath is then continued with the managementate so that motifies and hypotifiers with the dispositionistics on the managementate, and the said motifier with the dispositionistics on the managementate bound to said models and the occupant with the originate order to the core probe hypotidite with the obligatorizationist on the core probe hypotidite with the obligatorizationistics.

naconarticles

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72. The method of Chies 70 whorein and southin end is contented with the sunspectitions on that said studies unit deprintions with the objectnessession of the said southing and the content with the objectnesses of the souther with the objectness of the objectne

73. A method of detecting musleic and having at least two portions

A method of detecting nucleic and having at least two portio
 comprising:

providing a substrate having oligonucleotides attached thereto, the oligonucleotides having a sequence complementary to a first portion of the sequence of a musicio acid to be detected;

providing a compelho comprising at heat two types of manageration, each type of amoparticis having objected contents attracted thereo which are complementary to the objectmentation on a least care colour type of encoparation, the neoperation of the aggregate probe being board to each other as a result of the hybridization of the objectmentations are artered to them.

providing a type of linking oligonocteoides comprising a sequence
complementary to a second portion of the sequence of said muclei acid and a sequence
complementary to a portion of the sequence of the oligonocteoides attached to at loan
one of the types of sanoparticles of the core probe;

contacting and seated, said, the finding objects tool fact, the mithratie and the core probe under confliction effective to allow hybridization of said motion allow and the designation of the substants and to allow bybridization of the objects of

observing a detectable change.

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- 74. The neethed of any one of Claims 70-73 wherein the substants has a plannity of types of oligonuc/pointies attached to it in an array to allow for the detection of emiliple portions of a single molelo sold, the detection of multiple different nucleic sold, the detection of multiple different nucleic sold, the detection of multiple different nucleic solds, evolutions.
  - The method of any one of Claims 70-73 wherein the substrate is a transparent substrate or an opeque white substrate.
- 76. The method of Claim 76 wherein the detectable change is the formation of dark areas on the substance.
- The method of any one of Claims 70-73 wherein the nanoparticles in the core probe are made of gold.
- 78. The method of any one of Claims 70-73 wherein the substance is contacted with a sliver stain to produce the detectable change.
- The method of any one of Claims 70-73 wherein the detectable, change is
   observed with an optical scanner.
  - \$0. A method of detecting a modele acid having at least two portions comprising:
  - providing nanopurisles having oligomethroldes suitched thereo; providing one or more types of binding oligomethroldes, each of the binding oligomethroldes having two postions, the sequence of one portion being complementary to the sequence of one of the postions of the survivie soil and the sequence of the other postion being complementary to the sequence of the

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oliganucleotides on the nanoparticles,

- controling the nanoparticles and the binding diagonestocides under conditions officiality to allow hybridization of the alignmentations on the manoparticles with the binding diagonetic cides;
- contacting the models noted and the binding oligomacleotides under conditions effective to allow hybridization of the binding oligonucleotides with the mucleic solid; and
  - observing a detectable change.
- The method of Claim 80 wherein the nanoparticles are contacted with the binding oligonucleoides prior to being contacted with the auctrio acid.
- A method of detecting a nucleic sold having at least two portions occapiting:
- 15 providing nanoparticles having oligonucleotides attached thereto;
- providing one or more binding oligonucleotides, each of the binding oligonucleotides lawling two portions, the sequence of one protion being complementary to the sequence of the set has the opportune of the mother act dut at the sequence of the other portion being complementary to the sequence of the object-leotides on the
- contacting the annequations and the binding obgenucleotides under conditions offective to allow hybridization of the oligonucleotides on the nanoparticles with the binding objenucleotides,
- conditions offercive to allow hybridization of the binding oligonucleosides under 25 conditions offercive to allow hybridization of the binding oligonucleosides with the nucleic acid; and

observing a detectable change.

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- 83. A resulted of detecting nucleic axid having at least two portions comprising:
- contracting the nucleic acid with at least two types of particles having oligonacleotides attached thereto,
- the objectuationides on the first type of particles having a sequence complementary to a first portion of the sequence of the nucleic acid and being labeled with an energy donor,
- the oligonuclocities on the second type of particles having a sequence complementary to a second pertion of the sequence of the nucleic sold and being labeled with an energy acceptor.
  - the contacting taking place under conditions effective to allow hybridization of the oligonacteolides on the particles with the nucleic acid; and
  - observing a detectable change brought about by hybridization of the obsormolectides on the particles with the medicic sold.
- 84. The method of Claim 83 wheelth the energy donor and acceptor are Successori molecules.
- 85. A method of detecting models and having at least two portions 20 comprising:
  - providing a type of microspheres having oligonucleotides attached thorsto, the oligonucleotides baving a sequence complamatary to a first portion of the sequence of the motion acid and being labeled with a fluorescent molecule;
- providing a type of nanopariotes having oligomotivoides attached thereto,
  the oligomotivoides having a sequence complamentary to a second position of the
  sequence of the moticio sold, nanopariotes being capable of producing a describble
  change:

contacting the nucleic acid with the nucrospheres and the nunoparticles

ECLIANIMI 140

under conditions effective to allow hybridization of the oligonouslentides on the microspheres and on the nanoparticles with the methot acid; and observing a change in fluorescence, unother detectable change produced

by the nanoparticles, or both.

- 86 The method of Claim 85 wherein the detectable change produced by the nanoparticles is a change in color.
- 87. The northod of Claims 85 wherein the microsphores are latex microsphires 10 and the meroparticles are gold manaparticles, and changes in fluorescence, color or both are observed.
- 88. The notheod of Claim 87 factors competiting plating a portion of the unknown of the lates microsphases, asseparables and nucleic acid in an electration set part is located on an interoperous assertant part is proposed assertant in contract to make a proposed part of the prop
- 89. A method of detecting nucleic sold having at least two portions 20 comprising.

providing a first type of metallic or semiconductor assespanticles having oligonucleoxides attached thereto, the oligonucleoxides having a sequence complementary to a first pection of the sequence of the mulatic sold and being labeled with a fluorescent

peoryáling a second type of extallie or semiconductor nanoyenteles having oligocosolocides attached threeto, the oligococlocides having a sequence complementary or a second portion of the sequence of the nucleic sold and being labeled with a flavormost motoroid:

WO 03/51645

5

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coalecting the nucleic acid with the two types of nanoparticles under coaditions effective to allow hybridization of the oligonucleotides on the two types of nanoparticles with the nucleic acid; and

#### observing changes in fluorescence.

- 90. The mothod of Claim 89 further compraints photons a portion of the minimum of the autoparticles and nutcleir acid in an observation area located on a microproces material, treating the microproces material, treating the microproces material to as to resource any unbound manaparticles from the observation area, and then observed the denses in filteresource.
- 91. A method of detecting nucleic acid having at least two partiers comprising:
- providing a type of particle having oligonucleolides attached thereto, the oligonucleolides buring a first portion and a second portion, both portions being complementary to portions of the sequence of the audici acid;
- growfuling a type of prothe ollipsometroliders compelating a fault portions and a second portion, the first portion having a sequence complementary to the first portion of the ollipsometroliders standard to the particular and both portions being complementary to protein sof the sequence of the section soid, the probe oligenucleotides further being the labeled with a reporter moderate and one each.
  - contacting the particle and the probe oligonucleotides under conditions effective to allow for hybridization of the oligonucleotides on the particles with the probe oligonucleotides to preduce a satellite probe;
- then contacting the satellité probe with the puchoic acid under conditions

  25 effective to provide for hybridization of the nucloic acid with the probe obgonucleotides;

  removing the particles; and

detecting the reporter molecule.

WO OMS 1065

PCDESHALL 199

- The method of Claim 91 wherein the particles are magnetic and the reporter molecule is a fluorescent molecule.
- The method of Claim 91 wherein the particles are magnetic and the
   reporter molocule is a dyn molecule.
  - 94. The method of Claim 91 whereis the particles are magnetic and the reporter nucleoale is a redox-active molecule.
- 10 95. A kit comprising at least one custriener, the continuer holding a composition comprising at least two lops of ransporticise burings objective comprising an actual of themse, the adjustanceasions on the first type of accuration lawring a sequence complementary to the sequence of a first portion of a mobile soil, the objective risk the tensor type of natural prescribed lawring a sequence complementary to the sequence of 5 a second portion of 6 fee mobile soil.
  - 96. The kit of Claim 95 wherein the composition in the container further comprises a filler oligomelouide having a sequence complementary to a deed portion of the nucleic axid, the first portion being located between the first and second portions.
    - 97. The kit of Claim 95 wherein the ranoparticles are made of gold.
    - 98. The kit of Claim 95 further comprising a solid surface.
  - 99. A kit comprising at least two containers,
  - the first container holding nanoparticles having oligonucleotides attached thereto which have a sequence complementary to the sequence of a first pertion of a sucheic used, and

WO 03/51665

PCT/USUL/U1990

the second container holding nanopuritoes having of genuedoodides attached thereto which have a sequence complementary to the sequence of a second portion of the nucleic acid.

- 100. The kit of Claim 99 comprising a third container holding oligonucloodids having a sequence complementary to a third portion of the nucleic acid, the third portion being located between the first and second portions.
  - 101. The kit of Claim 99 wherein the ranoparticles are made of gold.
  - 102. The kit of Claim 99 further comprising a solid surface.
  - 103. A kit comprising at least two containers,
- On first container holding numparticles having oligonucleotides attached

  15 thereto which have a sequence complementary to the sequence of a first portion of a

  binding oligonucleotide, and
- the second container holding one or more types of binding one of more types of binding objected to the control of the control portion being complementary to the sequence of the collegerative of a portion of a multicide control portion being complementary to the sequence of a portion of a multicide control portion being complementary to the sequence of a portion of a multicide control portion being complementary to the sequence of a portion of a multicide control portion being complementary to the sequence of a portion of a multicide control portion being complementary to the sequence of a portion of a multicide control portion being complementary to the sequence of the control portion being complementary to the sequence of the control portion being complementary to the sequence of the control portion being complementary to the sequence of the control portion being complementary to the sequence of the control portion being complementary to the sequence of the control portion being complementary to the sequence of the control portion being complementary to the sequence of the control portion being complementary to the sequence of the control portion being complementary to the sequence of the control portion being complementary to the sequence of the control portion being complementary to the sequence of the control portion being complementary to the sequence of the control portion between the control po
- 104. The kin of Calina IOI which comprise additional containers, such holding as additional binding oligonochotisk, each additional binding oligonochotisk having a 23 separace comprising at least two persons, the fast professo being complementary to bit sequence of the oligonochotiske such as supersolated and the second portion being complementary to the sequence of one object professor for secondary and the second portion being complementary to the sequence of onesther portion of the noticed said.

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- 105. The kit of Claim 103 wherein the nanoparticles are made of gold.
- 106. The kit of Claims 103 further comprising a solid surface.
- 107. A kit comprising:

a consider balling one type of conspiration lawring edigeometrodes, and the fiberian and one concer types of Visiting diagnoscientists, each of the types of blanking edigeometrodes, but have been been completed as the complete and the complete

108. A kie compression ge losse use constantes, the constante holding metallist or is semicondustor manopuriciose having oligorastendoses stratabod theresto, the aligorasticolotica laving a sequence complementary to a portino of a susidise and send having fluorescent molecules attached to the ends of the oligorascitorioties part astached to the manopacities.

- 0 109. A kit comprising:
  - a substrate, the substrate having attached thereto asseputities, the sampasticles baving oligonacleotistes attached thereto which have a sequence complementary to the sequence of a first pertion of a rueleic sold; and
- a first consister holding manoparticles having oligonucleotides attached
  thereto which have a sequence complementary to the sequence of a second portion of the
  - 110. The kit of Claim 109 further comprising:

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a second container holding a binding oligonus/cetide having a selected sequence having at least two portions, the first portion being complementary to at least a position of the sequence of the oligonus/cetides on the nanoparticles in the first container;

- a third container holding nanoparticles having oligonucleotides attached themsto, the oligonucleotides having a sequence complementary to the sequence of a second portion of the binding oligonucleotide.
  - 111. A kit comprising at least three containers:

the first container holding nanoparticles;

the second container holding a first oligonucleutide having a sequence complementary to the sequence of a first portion of a nucleic zoid; and

the third container holding a second ofigurundentide having a sequence complementary to the sequence of a second portion of the models sold.

- 112. The kit of Chaim 111 farther comprising a fourth container holding a third oligonucleotide having a sequence complementary to the sequence of a third portion of the nucleic acid, the third portion being located between the first and second portions.
- 113. The kit of Claim 111 further comprising a substrate.
  - 114. The kit of Claim 113 further comprising:
- a fourth consister bolding a binding oligonucleotide having a scienced sequence having or least two portions, the first portion being complementary to at least a 25 portion of the sequence of the second eliginoidicolide; and
  - a fifth container holding an objectualisation having a requence complementary to the requence of a second portion of the binding objectuation.

WO 01/51/45

PCT/US0L/0156

- 115. The kit of Claim 111 wherein the oligonucleotides, managerticles, or both bear functional groups for stachment of the oligonucleotides to the nanoperticles.
- 116. The lot of Claim 113 wherein the substrate, nanoparticles, or both bear 5 functional groups for attackment of the nanoparticles to the substrate.
  - 117. The kit of Claim 113 wherein the substrate has nanoparticles attached to it.
  - 118. The kit of Claim 111 wherein the usnoparticles are made of gold.
  - 119. A kit compensing:

- a substants having oligonosleobides attached thereto which have a sequence complementary to the sequence of a first portion of a modele sold;
- a first container holding annoparticles having oligonecleotides attached

  thereto, some of which have a sequence complementary to the sequence of a second
  portion of the pucket solet, and
  - a second container bodding assopaticles having oligonucleotides atmohad thateto which have a sequence complementary to at least a portion of the sequence of the oligonucleotides attached to the assoparticles in the first container.
    - E20. A kit comprising:
      - a substrate;
      - a first container holding nanoparticles;
- a second container holding a first oligorauchoride having a sequence
  25 complementary to the sequence of a first pertion of a madeic solid;
  - a third container holding a second oligenselectide having a sequence complementary to the sequence of a second portion of the modelo noid; and
    - a fourth container holding a third oligonucleotide having a sequence

WO 91/51045

PCDUSHADI 190

#### complementary to at least a portion of the sequence of the second oligonableotide.

- 121. The list of Claim 120 wherein the oligonucleotides, nanoparticles, substrate or all bear functional groups for attachment of the oligonucleotides to the 5 nanoparticles or for attachment of the oligonucleotides to the adjustment.
  - 122. The kit of Claim 120 wherein the nanoparticles are made of notd.
  - 123. A kit comprising:
- a substate having oligomucleotides attached thereto which have a sequence complementary to the sequence of a first portion of a nucleic acid;
  - a first container holding liposomes baving oligonuclocides attached thereto which have a sequence complementary to the sequence of a second portion of the sequence old a set
- a success container holding naneparticles having at least a first type of .

  oligatuc-leotides attached thereto, the first type of oligonucleotides having a hydrophobic group attached to the end not attached to the ranoparticles.
  - 124. The kit of Claim 123 wherein:
- the nanoparticles in the second container have a second type of oligental-toticles attacked thereto, the second type of oligonuclocides having a sequence complementary to the sequence of the oligonuclocides on a second type of sunoparticles; and the kit further comprises:
- a third centainer holding a second type of nanopanioles having 25 oligonautotides statisfied thereto, the eligonautotidides having a sequence compliamentary to at least a portion of 6s sequence of the second type of oligonautocides on the first type of nanoparticles.

PCY/USBL/DI 190

#### 125. A kit comprising:

a substrate, the authorists having attached thereto nanoparticles, the nanoparticles having oligonucleoxides ninoched thereto which have a sequence complementary to the sequence of a first portion of a nucleic cold; and

a first container belding an aggregate probe: comprising at least two types of manoparticles through objects attended thereto, the manoparticles of the aggregate probe policy bused to see that ear a setual of the hybriditation of some office objects on the control of them, at least one of the types of manoparticles of the aggregate; probe having objects detailed thereto which have a requirement of the control of the complementary to asked patient of the sequence of the motion is not.

#### 126. A kit comprising:

a substrate, the substrate having oligonucleorides attached thereto, the
 oligonucleorides having a sequence complementary to the sequence of a first portion of a
 substrate acid; and

a first consistent bolding om aggregate proble competiting at these two types of annoporations having oligementeedies attended factors, the unequalities of the aggregate proble place for each design at a result of the high-pictifications of some of the obligence/continued anticabel to them, as least one of the types of manoparticles of the 20 aggregate proble having objected-entities statistical thereto which have a sequence complementary to accomplementary the accomplementary to acc

127. The left of Claim 126 wherein the substrate has a plorality of types of oligonuclionides attached to it is an army to allow for the detection of multiple portions of a single crucleic acid, the detection of multiple different nucleic acids, or both.

# 128. A kit comprising:

a substrate having oligonucleotides attached thereto;

WO 93/51/65

.....

a first continue bedding an agarystic probe comprising at least two types of autoparticles having oligonethoddes stached direce, the autoparticles of the agarystapes probe large lowed to each direct as a result of the bipdistante of zone of the oligonetheoides attached to files, at least one of the types of autoparticles of the 5 aggregate probe having oligonethooides attached files the which have a sequence consumentative, on the protect of the sequence of the mixed-said, and

a second consider holding susceptibles beying at least two types of oligonucleolides standard thereon, the first type of oligonucleolides baseling a sequence consupercentary to a second perion of the sequence of the studies and, and the second to type of oligonucleolides baseling a sequence complementary to at least a portion of the sequence of complementary to at least a portion of the sequence of colligonucleolides baselined to the substrate.

## 129. A kit comprising:

- a substrate, the substrate barring oligonucleotides attached theoreto, the 15 oligonucleotides having a sequence complementary to the acquence of a first portion of a
  - u first container holding liposomes having oligonucleotides attached thereto which have a sequence complementary to the sequence of a second portion of the modelec acid; and
- a second container holding an aggregate probe comprising at least two
  types of measurables thriving obligomedecides attached threats, the amountaines of the
  aggregate purched by bound to end-these as a restart of the hybridistion of some of the
  objective of the container of the
  - 130. The kit of any one of Claims 125-129 Wherein the substrate is a transparent substrate or an opeque white substrate.

WO 01/51/65

PCT/USUL/U1798

- The left of any one of Claims 125-129 wherein the nanoparticles of the aggregate probe are made of gold.
- 132. A kit comprising at least three containers:
- the first container holding nanoparticles;
- the second container holding a first oligonucleotide having a sequence
- complementary to the sequence of a first portion of a nucleic sold; and
  the third container holding a second oligonucleotide having a sequence
- 10 complementary to the sequence of a second portion of the michie acid.
  - 133. The kit of Claige 132 further comprising a fourth container holding a third oligonucleotide having a sequence complementary to the sequence of a third portion of the musicie sold, the third portion boing located between the first and accord portions.
    - 134. The kit of Claim 132 further comprising a substrate.
    - 135. The kit of Claim 134 further comprising:
- a fourth container holding a binding of genucleotide having a selected
  sequence having at least two portions, the first portion being complementary to at least a
  partice of the sequence of the second objectualistics, and
  - a fifth container bolding an oligonucleotide having a sequence complementary to the sequence of a second portion of the binding oligonucleotide.
- 15 136. The kit of Claim 132 wherein the disconnelectides, nanoparticles, or both bear functional groups for attachment of the oligonucleotides to the nanoparticles.
  - 137. The kit of Claim 134 wherein the substrate, nanoparticles, or both bear

WO 03/51665

CCDUSHIDI 199

functional groups for situationent of the nanoparticles to the substrate.

- 138 The kit of Claim 134 wherein the substrate has nonoparticles attached to it.
- 139. The kit of Claim 132 wherein the nanoparticles are made of gold.
- 140. A kit comprising:
- a substitute having oligonacleotides attached thereto which have a sequence complementary to the sequence of a first portion of a nucleic scid;
- a first consister holding manoparticles having oligonucleosides attached thereto, some of which have a sequence complementary to the sequence of a second portion of the suelicit zeid; zod
- a success container holding nanoperticles having oligorordeoisées attached
  thereto which have a sequence complementary to at least a portion of the sequence of the
  oligorousloobides attached to the reneparticles in the first container.
  - 141. A kit comprising:
    - a substrate;
  - a first container holding nanoparticles;
- 20 a second container holding a first oligonucleotide having a vequence complementary to the ecquence of a first portion of a nucleic acid;
  - a third container holding a second oligonucleotide having a sequence
- complementary to the requence of a second portion of the nucleic sold; and
  a fourth container holding a third objective-leaded lawing a sequence
  25 complementary to at least a portion of the sequence of the second objects
  - 142. The kit of Claim 141 wherein the oligonucleotides, nanoparticles, substrate or all bear functional groups for attachment of the oligonacleotides to the

WO 91/51665

PCT/QSRL/ULTSU

#### nanoparticles or for attachment of the oligonucleotides to the substrate.

- 143. The kit of Claim 141 wherein the nanoparticles are made of gold.
- 5 J.44. A kit comprising:
  - a substrate having oligonucleotices attached thereto which have a sequence complementary to the sequence of a first portion of a resolute soid,
- a first container holding liposomes having oligonucleotides attached
  thereto which have a sequence complementary to the sequence of a second parties of the
  macleic acid; and
  - a second container holding metopacticles having at least a first type of oligonucleotides attached thereto, the first type of oligonucleotides having a hydrophobic group attached to the end not attached to the nanoparticles.
- 5 [45. The kit of Claim 144 wherein:

the nazoparticles in the second container have a second type of oligonucleoidse attathet diserte, the second type of oligonucleoidse having a sequence complementary to the sequence of the oligonucleoidse on a second type of samoparticles; and the life further comprise:

- 20 e third consider hobbing a second type of nanoparticles having oligeneristicities statished threats, the oligeneristorides having a sequence complementary to at least a portion of the exquence of the second type of oligenreclosides on the first type of nanoparticles.
  - 146. A kit comprising at least two containers.

the first container bodding particles having oligonacteristics attached theceto which have a sequence complementary to the sequence of a first portion of a mucleto acid, the oligonacterodies being labeled with so energy donor on the ends not

WO 93/51665

PCT/USIL/IV 190

attacked to the particles,

the second consistent habiting particles having olligeneedensides attached thereto which have a sequence complementary to the requence of a second portium of a musticle cold, the olligeneedensides being labeled with an energy acceptor on the ends not statehold of the particles.

- 147. The kit of Claim 146 wherein the energy donor and acceptor are fluorescent molecules.
- 10 146. A bit cougniting at least one contains, the container bolding a first space of particles having dispuncionables attained theses which have a sequence complementary to the expected of a flam price of the dispuncionables being babed with an except down on the ends out statistics to the particles, and a second byte of particles having dispuncionables entained denore which leave a sequence of the ends of the ends of the end of the e
- 149. The kit of Chaim 148 wherein the energy donor and acceptor are fluorescent molecules.
  - 150. A kit comprising:
  - a first container holding a type of microspheres baving oligonucleosides attached thereto, the oligomeshooides baving a sequence complementary to a first portion of the sequence of a nucleic acid and being labeled with a fluorescent molecule; and
- a second container holding a type of nanoparticits brying oligosurcteorides attached thereto, the oligonucleorides baving a sequence complementary to a second portion of the sequence of the suchtic acid.

WU 01/51665

15

PCT/US01/01190

- 151. The kit of Claim 150 wherein the microspheres are letter microspheres and the nanoparticles are gold nanoparticles.
  - 152. The kit of Claim 150 further comprising a microporous material.
  - 153. A kit comprising:

a first container habling a first type of metallic or semiconductor amogaritales having oligonusbosidies attached thereto, the oligonusbosides having a sequence complementary to a first portion of the sequence of a nucleic acid and being this labeled with a fluoressent molecule; and

a second container holding a second type of metallic or sensiconductor nanoparticles having oligonecteotides statesthal thereto, the oligonecteotides having a sequence completeneity to a second perhan of the sequence of a naciela said and bring habeded with a flavorescent molecole.

- 154. The kit of Claim 153 further comprising a relateporous material.
- 155. A lek comprising a container holding a satellite probe, the satellite probe
- a particle having attached thereto oligomoleotides, the oligomoleotids having s first portion and a second portion, both portions having sequences complementary to postions of the sequence of a nucleic acid; and
- probe oilgenunleodiste hybridised to the oilgenunleodises subschola to the anonparticine, the pube oilgenunleodiste having a first profices and a second portion, the 25 first portion having a sequence complementary to the sequence of the first profice of the oilgenunleodiste statched to the particles, both proficers having exquences complementary to perform of this sequence of the secoles acid, the pube oilgenunleodiste further having a reporter molecular school to our end.

PC DESERTED 191

- 155. A kii comprising a consistent holding an aggregate probe, the aggregate probe comprising at least two types of emorphisish laving objicomelections attached thereth, the emorphisish of the aggregate probe being beauted to och days as a small of 5 the hydridization of some of the object-enclosides unknowled to them, at least one of the types of assuspectation of the aggregate probe having objected state-state therether which have sequence complementary to period of the recoperate of a contein said.
- 107. A kit comprising a container holding an appropria probe, the apprepria probe comprising at least two types of emospaticials having eligentalenties standard thereon, be encountried of the appropriate of his proprietal probe having value to each other as a world of the hybridization of some of the oligomorboides attended to them, at least one of the types of annoparation of the appropriate probe having oligomorboides attended thereto which have hybripholic joiner standards to the end not attended to the management.
- 1.58. An egyregate probe, the agyregate probe comprising at least two prote of nanoparticles having oligosociandae attached thereto, the assopatation of the aggregate probe. being board to each other as result of the hybridisation of towar of the oligosocianidae attached to them, at least one of the types of amorpative of the oligosocianidae attached to them, at least one of the types of amorpative of the oligosocianidae periods of the oligosocianidae attached to the oligosocianidae of the oligosocianidae attached to the oligosocianidae of the oligosocianidae oligosoci
- 119. The aggregate peaks of Claim 134 comprising two types of anoquaristics each having two types of edigenestocides attached thereto, the first type of 20 obgenutionides standard to each type of immorphiside having a expense consplanation to a portion of the requirement of a motion soft (in a sound type of disappeaksonides absoluted to the first type of sanosparticles having a sequence complanations yet of the protein of the sequence of the second type of objenutive type of the sequence of the second type of objenutive type of the sequence of the second type of objenutive type of the sequence of the second type of objenutive type of the sequence of the second type of objenutive type of the sequence of the second type of objenutive type of the sequence of the second type of objenutive type of the sequence of the second type of objenutive type of the sequence of the second type of objenutive type of the sequence of the second type of objenutive type of the sequence of the second type of the second type of the sequence of the second type of the second

PCT/USHAD 150

### of nanopartities

- 10. The aggregate point of Claim 15 comprising their types of emagneticities bening diginacticities standed there, the alignmentative amount of the different of the subgranticities bening a separate complementary to these a portion of the requires of the singuracticities thereign a separate complementary to a transporticitie, the obligamentarities standards the backword type of compressional barings a separate complementary to it is an a portion of the separate complementary to it is an appearance of the fact type of compressional barings a separate complementary to a fact type of managementation baring the type of compressional barings to specific confidence of a standard factors, the fact type of dispundenticities having no specific coffee expected of associated barings of the second type of dispundentiable the type as separate complementary to a tester a position of the sequence of the dispundential baring a segarous complementary to a tester a position of the sequence of the dispundential barings and standard on the fact or round type of emographics.
- 15 161. An agaregate peode, the agaregate probe comprising at least low open of measuration having ollogomotorides attached thereto, the embapticides of the agaregate probe being load to each other as a stream of the hydraticides of smoot of the offigureutosides attoched to them, at least one of the types of annoquenticide of the offigureutosides attoched to them, at least one of the types of annoquenticide of the agaregates probe having ollogomotosides attached thereto which have a hydrophobic against the end out statehed the managerificide.
- 162. A kit comprising a contribute holding a core probe, the core probe comprising at test two types of annoparticles throng oligonoxicotides attached thorses, the transparticles of the core probe being bound to each other as a result of the hybridization of source of the oligonucleotides attached to thron.
  - 163. The kit of Chrim 162 further comprising a substrate having oligonuclootides attached threeto, the oligonucleotides having a sequence complementary to a first previous.

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## of the sequence of a mucleic acid to be detected.

- 164. The lit of Claim 162 or 163 further comprising a constance hedding a type of manageriation having two types of ollogonaclocidios attended thereos, the first type of ollogonaclocidios through a exequence complementary or a second periodic of the medicial scid, and the second type of ollogonaclocidios having respector complementary to a position of the sequence of the ollogonaclocidios sharing respector complementary to a position of the sequence of the ollogonaclocidios sharing respector complementary to a position of the sequence of the ollogonaclocidios sharing respector complementary to a position of the sequence of the ollogonaclocidios sharing respector.
- 165. The his of Cities 180 or 185 feether comprising a consister building a type of linking objected ordinates expertising a sequence complementary as exceed profits of the sequence of the residence of the authorities and an assume complementary to a position of the sequence of the digaranticologies attached as at least one of the types of manuparticles of the one public.
  - 166. A core probe comprising at least two types of nanoparticles having oligonucleotics attached thereto, the nanoparticles of the core probe being bound to each other as a result of the hybridization of some of the oligonucleotides attached to these.
- 20 167. A substrate having nanoparticles attached thereto.
  - 168. The substrate of Claim 167 wherein the manoparticles have oligopoutcorities attacked thereto which have a sequence complementary to the sequence of a first pertion of a pucheic soid.
  - 169. A metallic or semiconductor paraparticle baving oligonucleotides attached thereto, the oligonucleotides being labeled with fluorescent molecules at the ends not attached to the remoparticle.

PCT/USDE/01190

### 170. A satellite peobe comprising:

a particle having attached thereto elligenzatestides, the oligenzatestides, the oligenzatestides having a first portion and a second portion, both portions having sequences a complementary to portions of the sequence of a nucleic skill, and

probe oligonucleosides hybridised to the oligonucleosides stateded to the oligonucleosides stateded to the energetides, the probe oligonucleosides having a filt protein and a second portion, the first portion is but a second portion, the first portion is but a second portion, and the probes of the filter portion of the filter portion of the filter portion of the oligonucleosides stateded to the parallels, both positions having sequences complementary to to protein sections of the secondary of t

## 171. A method of nanofibrication comprising

providing at least one type of linking oligenacleotide having a selected sequence, the acquence of each type of linking oligenzeleotide having at least two

providing one or more types of manaparticles having oligomucleosides untuched thereo, the oligomucleosides on each of the types of manaparticles having a sequence complementary to the sequence of a portion of a histing oligomucleosides; and contacting the thinking oligomucleosides and nanaparticles under conditions

effective to allow hybridization of the offgeomorleotides on the nanoparticles to the linking oligonactionides so that a desired nanopasterial or assostanceuro is formed wherein the nanoparticles are held together by offgeomorleotide connectors.

172. The method of Chies 171 whorein at least two types of managarticles having oligonuclosides extended finered are provided, the oligonuclosides on the first yes of antopauticles having a sequence complementary to a first portion of the acquance at linking oligonucloside, and the oligonucloside on the second type of managertields.

PCTYUSBURD 194

having a sequence complementary to a second portion of the sequence of the linking oligonucleofide.

- 173. The method of Claim 171 or 172 wherein the zanoparticles are metallic 5 nanoparticles, semiconductor nanoparticles, or a combination thereof.
  - 174. The method of Claim 173 wherein the moultic panoparticles are made of gold, and the semiconductor nanoparticles are made of C6SelZnS (core/shell).
- 175. A method of nanofabrication comprising:
- providing at least two types of nanoparticles having oligonucleotides
- the oligopucleotides on the first type of nanoparticles having a sequence complementary to that of the oligonacleosides on the second of the nanoparticles,
- the oligonucleotides on the second type of nanoparticles having a sequence complementary to that of the oligonucleotides on the first type of nanoparticles; and
- contacting the first and second types of nanoparticles under conditions effective to allow hybridization of the oligonucleotides on the nanoparticles to each other 20 so that a desired nanoquaterial or nanostructure is formed.
  - 176. The method of Claim 175 wherein the nanoparticles see metallic nemenanticles, semiconductor nanoparticles, or a combination thereof.
- 177. The mothod of Claims 176 wherein the metallic nanoparticles are made of gold, and the semiconductor nanoparticles are made of CdSe/ZeS (core/shell).
  - 178. Nanomaterials or nanostructures composed of nanoparticles having

PCTAGOUAN 190

obigonreciecides attached thereto, the nanoparticles being held together by obigonucleotide connectors.

- 179. The parameterials or parameterises of Claim 178 wherein at least some of 5 the oligonoclootide connectors are triple attanded.
- 180. The manometerials or eacostructures of Claim 178 wherein the nanoparticles are metallic nanoparticles, semiconductor nanoparticles, or a combination flavoral.
- The nanoconteinies or measurectures of Claim 180 wherein the metallic sanoparticles are made of gold, and the semiconductor manoparticles are made of Concentrations.
- 15 12. A composition consprising at frest two types of nanoparticles having objective-forces, the objective-force in the first type of nanoparticles having a supremoted complementary to the supremo of a final calculation of a function and or a final calculation of a function and or a final calculation of a function and or a final calculation of a function of the supermote complementary to the sequence of a second portion of the nucleic cold or objective for the function of the nucleic cold or objective forces.
  - 183. The composition of Claim 182 wherein the nanoparticles are metallic nanoparticles, semiconductor nanoparticles, or a combination thereof.
- 184. The composition of Claim 183 wherein the motallic nanoparticles are useds of gold, and the semiconductor nanoparticles are made of CdSetZaS (core/stell).
  - 185. An assembly of containers comprising:

account at the

a first container holding nanoparticles having oligonucleotides attached

a second container holding manaparticles having oligonacteotides attached

thereto,

the oligenucleosides anathrid to the nanoparticles in the first constinct baving a sequence complementary to that of the oligenucleotides attached to the unnegatities in the second container, the oligenucleotides attached to the manoparticles in the second container.

having a sequence complementary to that of the oligomorphic standed to the nanoparticles in the second contenter.

186. The assembly of Claim 185 wherein the unreparticles are metallic annoparticles, semiconfuctor nanoparticles, or a combination thereof.

187 The assembly of Claim 186 wherein the mostlio nanoparticles are made of gold, and the acmiconductor nanoparticles are made of CoSe/ZnS (core/thell).

188. A nanoparticle having a plurality of different eligopucleotides alterbed

189. A method of separating a selected models wild having at least two portions from other models aside, the method comprising:

providing two or more types of nanoparticles having oligonucleotides analoched therete, the oligonucleotides on such of the types of nanoparticles baving a sequence complementary to the supersect of our of the portions of the selected nucleoracid; and

occurating the nucleic acids and nunoparticles under conditions effective to allow hybridization of the oligonaclootides on the nunoparticles with the selected

PCDUSHIBI 191

nucleic acid so that the neroperticles hybridized to the selected nucleic acid aggregate and precipitate.

- 190. A method of binding oligometrotides to charged nanoparticles to produce
- 5 stable nanoparticle-oligonacioside conjugates, the method comprising: providing oligonacionides having covatently bound thereto a moiety comprising a functional group which can baid to the nanoparticlet;
- contacting the oligonusteetides and the nanoparticles in water for a period of time sufficient to allow at least some of the oligonusteetides to bind to the nanoparticles;
  - adding at least one sait to the venter to form a sait solution, the lottle strongsh of the salt actual being sufficient to overcome at least partially the electrosteric attraction or regulation of the oligonucleotides for the assoparticles and the electrosteric expulsion of the oligonucleotide for each other; and
- 15 contacting the eligentucleotides and nanoparticles in the rait solution for an additional period of time sufficient to allow sufficient additional origonucleotides to bind to the nanoparticles to produce the elable nanoparticle-oligonucleotide sunjugates.
- 191. The method of Claim 190 wherein the nanoparticles are metal 29 nanoparticles or semiconductor nanoparticles.
  - 192. The method of Claim 191 wherein the nanoparticles are gold nanoparticles.
- 15 193. The method of Claim 192 wherein the moiety comprising a functional group which can bind to the comparticles is an allemethiol.
  - 194. The method of Chim 190 wherein all of the sult is added to the water in a

WO 93/51668

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### sinule addition.

- 195. The mothod of Chairs 190 wherein the salt is added gradually over time.
- 19%. The method of Claims 190 whereis her said is aclored from the groupconsisting of sodium chinride, mergenium-chicride, potentium chinride, aumonium, chicride, sodium, sozzate, aumonium scetate, a combination of two or more of these saits, nor of these saits in a phosphate buffer, and a combination of two or more these saits as a phosphate buffer.
- 197. The method of Claim 196 wherein the soit is sediram chicride in a phosphate buffer.
- 198. The method of Claim 190 wherein annoparticle-digoaucleotide 15 conjugates are produced which have the objectivelest greated on surface of the nanoparticles at a surface density of at least 10 picomolesten?.
  - 199. The method of Claim 198 wherein the oligonucleotides are present on surface of the resoperticles at a surface density of at least 15 picomoles/on<sup>2</sup>.
  - 200. The method of Claim 199 wherein the oligonucleotides are present on surface of the nanoparticles at a surface density of from about 15 picotooles/cm² to about 40 picomoles/cm².
- 25 201. A method of binding ofigoraucleotides to nanoparticles to produce nanoparticle-oligonaucleotide conjugates, the method comprising:
  - providing alignmucleatides, the alignmucleatides comprising at least one type of recognition alignmucleatides, each of the recognition alignmucleatides comprising

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a spacer portion and a recognition portion, the spacer portion being designed so that it can bind to the nanoparticles; and

- contacting the oligonucleosides and the manaparticles under conditions
  effective to allow at least some of the recognition oligonucleosides to bind to the
  sampparticles to produce the nanoparticle-oligonucleotide conjugates
- 202. The method of Claim 201 wherein each of the spacer positions of the recognition oligonucleotides has a mosety covalently bound thereto, the anoisty comprising a functional group which can bind to the nanoparticles.
- comparising a functional group which can beind to the instrupanticles.

  200. The method of Claim 201 wherein the managarticles are metal anapparticles or semiconductor managarticles.
- 204. The method of Claim 203 wherein the nanoparticles are gold
- 205 The method of Claims 204 wherein the spacer portion comprises at least about 10 guelectides.
- 20 206. The method of Claim 205 wherein the spacer portion comprises from about 10 to about 30 muclootides.
- 207. The method of Claim 206 wherein the bases of the nucleotides of the spacer are all adentities, all thyunines, all cytosines, all uracits, or all guarannes.
  - 208. A method of biasing oligonucleotides to transparticles to produce nanoparticle-eligonucleotide conjugates, the method comprising: providing oligonucleotides, the oligonucleotides congrising:

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## a type of recognition oligonucleotides; and a type of diluent oligonucleotides;

contacting the oligenucleatides with the nanoparticles under conflicing
effective to allow at least some of each of the types of oligenucleotides to bind to the
manaparticles to produce the nanoparticle-oligonucleotide conjugates.

- 209. The method of Claim 208 wherein the nanoparticles are motal nanaparticles or semiconductor nanoparticles.
- 10 210. The method of Claims 209 wherein the nanoparticles are gold transparticles.
- 211. The method of Claim 208 wherein each of the recognition oligonuclootides comprises a spacer portion and a recognition portion, the spacer portion is being designed to first it can bind to the manoparticles.
  - 212. The method of Chaira 211 wherein each of the spacer portions of the recognition oligonucleotides has a moiety covalently bound thereto, the moiety comperising a functional group which can bind to the nanoparticles.
  - 213. The method of Claims 211 wherein the spacer portions of the recognition oligomethodides econocises at least about 10 nucleotides.
- The molbod of Claim 213 wherein the spacer portions of the recognition
   oligometeotides comprises from about 10 readsortides to about 30 nucleotides.
  - 215. The method of Claim 211 wherein the bases of the nucleocides of the spacer are all nderines, all thymness, all eyesines, all tracils or all guanines.

PCT/05801/01150

- 216. The method of Claim 211 wherein the diluont oligonucleotides contain shout the same number of nucleotides as are contained in the spacer portions of the recognition oligonucleotides.
- 217. The method of Claim 216 wherein the sequence of the diluent oligonacleotides is the same as the sequence of the spacer portions of the recognition oligeauc)rotides.
- 218. The method of Chairs 20% wherein the oligonucleotides comprise at least two types of recognition oligonucleotides.
- 219. A method of binding oligonacheolides to charged nanoparticles to produce
- nanopacticle-obligamecleoble conjugates, the method comprising:

  providing oligamecleotides having covalently bound thereto a moisty comprising a functional group which can bind to the nanoparticles, the oligomacleotides comprising:

a type of recognition oligonucleorides; and

a type of diluent oligonocleotides;

contacting the oligonacleotides with the nanoparticles in water for a period of time sufficient to allow at least some of each of the types of oligonucleotides to bind to

adding at least one saft to the water to form a saft solution, the tonic strength of the selt solution being sufficient to overcome at least partially the electrostatic 25 attraction or repulsion of the oligonucleotides for the nanoperticles and the electrostatic repulsion of the oligonucleotides for each other; and

contacting the offgenucleofides and nanoparticles in the salt solution for so additional period of time sufficient to allow additional oligonucleotides of each of the

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types of oligonucleotides to bind to the nanoparticles to produce the nanoparticleoligonucleotide conjugates.

- 220. The method of Claim 219 wherein the nanoparticles are metal

  5 nanoparticles or semiconductor nanoparticles.
  - 221. The method of Claim 220 wherein the nanoparticles are gold nanoparticles.
- 222. The method of Claim 231 wherein the molety comprising a functional group which can bind to the nanoparticles is an alkanethiol.
  - 223. The method of Claim 219 wherein all of the salt is added to the water in a single addition.
    - 224. The method of Claim 219 wherein the solt is added gradually over time
- 225. The nuthood of Chains 219 wherein the salt is solected from the group consisting of sodium absisting, magnetism shoulds, potassism shoulds, memoration, 20 shoulds, solect, accompanion ascerts, a combination of two or same of these salts, so so of three salts in a phosphase buffer, and a combination of two or same of the salts in a phosphase buffer, and a combination of two or same these salts in a phosphase buffer, and a combination of two or same these salts in a phosphase buffer.
- 226 The roethod of Claims 225 wherein the salt is sedium chloride in a 25 phosphate buffer.
  - 227. The method of Claim 219 wherein nanopamiele-oligenmeteoide conjugates are produced which have the oligenucteorides are present on suction of the

PCT/USHA1194

nanoparticles at a surface density of at least 10 picomoles/cm<sup>2</sup>.

- 228. The method of Claim 227 wherein the oligonuclootides are present on surface of the passoparticles at a surface density of at least 15 picomoles/em².
- 229. The method of Claims 228 wherein the oligonacleotyles are present on surface of the nanoparticles at a surface density of from about 15 picomoles/cm² to shout 40 picomoles/cm².
- 230. The method of Claims 219 wherein each of the ecognition oligomedicentites comprises a spacer portion and a rosospeticles portion, the spacer portion lawing statched to it the coniesty comprising a functional group which can bind to the manoparticles.
- 5 231. The method of Claim 230 wherein the spacer portion comprises at least about 10 moderatides.
- 232. The method of Claims 231 wherein the spacer pection comprises from about 10 to about 30 multipudes.
- 233. The method of Claim 230 wherein the bases of the moleculdes of the spacers are all adequace, all thoraines, all cylosines, all unwills, or all gussines.
- 234. The motival of Claim 230 wherein the diluent oligenselections contain 25 about the same number of nucleotides as are contained in the spacer portions of the recognition oligonacieotides.
  - 235. The method of Claim 234 wherein the sequence of the diluent

PCT/USHAN 199

eligonucleotides is the same as the sequence of the spacer portions of the recognition of sequence of the spacer.

- 236. The method of Claim 219 wherein the alignmediatrides comprise at least
   two types of recognition olignmediatrides.
- 237. Nanoparticio-oligouçuloside conjugate which are nanoparticio having oligonezionidas attribat in hom, the oligonezionidas territorio attribat in hom, the oligonezionidas per present on surface of this assegnaticio at a surface dentiny sufficient so that the conjugates are stolin, at tenti some 10 of the oligonezionidas having a sequence complementariary to al least one portion of the sequence of a model insulf or substitution.
  - 238. The conjugates of Claim 237 wherein the eligentucleotides are present on author of the nanoparticles at a surface density of at least 10 piccondector.
  - 239. The nanoparticles of Classa 238 wherein the eligonactorides are present on surface of the nanoparticles at a surface density of at least 15 picomoleuters.
- 240. The nanoparticles of Claim 239 wherein the oligonucleoxides are present 20 on nurface of the nanoparticles at a surface density of from about 15 picomolectim<sup>3</sup> to about 40 picomolectim<sup>3</sup>.
- 241. The nanoparticles of Claim 237 whorein the nanoparticles are metal nanoparticles or senticonductor nanoparticles.
- The necoparticles of Claim 241 wherein the manaparticles are gold nanoparticles.

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- 243. Numperticles having oligonatoriotics metabol to them, the oligonatoriotides comprising at least one type of recognition oligonatoriotides comprising a least one type of recognition oligonatoriotides comprising a speer profiles and a recognition goalderies persion, the speers portion being designed to that it is bound to the assoparticles, the excognition 5 portion buring a texpense complementary to at least one pretion of the requester of a marches used or subtract opionatoriotide.
- 244. The nanoparticles of Claim 243 wherein the spacer portion has a moiety covalently bound to it, the moiety comprising a functional group through which the 
  10 spacer position is bound to the supoparticles.
  - 245. The nanoparticles of Claims 243 wherein the spacer portion comprises at least about 10 nucleotides.
- 15 246. The ranoparticles of Claim 245 wherein the spacer portion comprises from about 10 to about 30 sucleotides.
- 247. The manoperticles of Claims 243 wherein the bases of the nucleofides of the spacer portion are all adentices, all flyonines, all cytosines, all uracills or all guantnes.
- 248. The nanoparticles of Claim 243 wherein the oligonocloodides are present on surface of the compacticles at a surface density of at least 10 picomoles/cm².
- 249. The nanoparticles of Claim 248 Wherein the oligomicleotides are possent 25 on surface of the nanoparticles at a surface density of at least 15 picomoleakus.<sup>3</sup>.
  - 250. The encoparticles of Claim 249 wherein the oligonucleotides are present on surface of the nuneparticles at a surface density of from about 15 piscenoles/on<sup>2</sup> to

15

PCTMSSMAN 190

### about 40 picomoles/cm<sup>2</sup>.

- 251. The ranoparticles of Claim 243 wherein the nanoparticles are metal nanoparticles or sentronductor nanoparticles.
- 252. The method of Claim 251 wherein the nanoparticles are gold nanoparticles
- 253. Nanoparticles having oligonocleotides attached to them, the 10 oligonocleotides couprising:
  - at least one type of recognition oligonucleotides, each of the types of recognition oligonucleotides comprising a tequence complementary to at least one petion of the sequence of a nucleic acid or another oligonucleotide; and
    - a type of diluent oligonucleotides.
- 256. The assoprations of Class 233 wherein, each of the recognition oligomentoolists comprises a spacer portion and a recognition portion. Because from the spacer portion being designed so that it is bound to the assopration, the recognition portion hering a southern elegenate complementary to at least one portion of the sequence of a suction said or another oligomentoolists.
- 255. The nanoparticles of Claim 254 wherein the spacer portlen has a moisty covalently bound to it, the moisty comprising a functional group through which the spacer portion is bound to the nanoparticles.
- 256. The naneparticles of Chim 254 wherein the spacer portion comprises at least about 10 sucleotides.

WU 01/51665

PCT/USDL/01190

- 257. The nanoparticles of Claim 256 wherein the spacer portion comprises from about 10 to about 30 nucleotides.
- 258. The nanoparticles of Claim 254 wherein the bases of the nucleotides of the 5 spacer portion are all adecines, all thymines, all cytosines, all unacils or all guaranes.
  - 259. The nanoparticles of Claim 253 wherein the oligonostoolides are present on surface of the nanoparticles at a surface density of at least 10 picomolepions<sup>2</sup>.
- 260. The managarticles of Claim 259 wherein the oligomorleotides are present to on surface of the managarticles at a surface density of at least 15 picomoleotom<sup>2</sup>.
  - 261. The nanoparticles of Claim 260 wherein the oligonocheckées are present on surface of the nanoparticles at a surface density of from about 15 picomoles/cm<sup>3</sup> to about 40 picomoles/cm<sup>3</sup>.
  - 262. The nanoparticles of Claim 254 whorein the diluted eligensucleotides contain about the same number of queleotides as are contained in the spacer portions of the recognition oligonactosides.
- 20 263. The nanoparticles of Claim 262 wherein the sequence of the diluent obigonucleorides is the same as that of the spacer portions of the recognition obigonucleorides.
- 264. The nanoparticles of Claim 253 wherein the nanoparticles are motal 25 nanoparticles or senjocondator nanoparticles.
  - 265. The autoparticles of Claim 264 wherein the assopraticles are gold nanoparticles.

PCT/USHIAH 150

266. A method of detecting a nucleic acid comprising:

contacting the metals and with at least one type of nanoparticle-oligonucleoride conjugates according to any one of Chains 237-242 under conditions effective to allow bybridization of the oligonucleorides on the nanoparticles with the nucleic soid; and

observing a detectable change brought about by hybridization of the object-to-ides on the nanoparticles with the nucleic acid.

## 267. A method of detecting a method acid comprising:

- centacting the zustaic acid with at least one type of nanoparticles according to any one of Claims 241-265 under conditions effective to allow hybridization of at least one of the types of recognition alignonacloodeds on the nanoparticles with the metalo acid; and
- observing a detectable change brought about by hybridization of the recognition 15 observed cotides with the nucleic acid.
  - 268. A method of detecting a nucleic acid having at least two portions comprising:
- providing a type of menoparticle-oligonucleotide conjugates according to any one of Claims 237-242, the oligonucleotides on each unosparticle having a sequence
- complementary to the sequence of at lenst two portions of the nucleic crid; contacting the nucleic acid and the conjugates under conditions effective to allow hybridization of the objectoriodes on the nanoparticles with the two or more
- portions of the moteic soid; and

  25 observing a detectable change beought about by hybridization of the
  oligomolecules on the nanopartistes with the moteic acid.
  - 269. A method of detecting a mucleic sold linving at least two portions

PCT/USBILIDI 191

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causing the useful scient with a least two types of insperielical colorant-inference places according any one of Climan 273-60, the disputation/circle on the managearline of the first type of conjugates laving a sequence complementary to a Sell perfusion of the compares of the medic and, the eligopacellocide and the magneticies of the second type of conjugates always agreement complementary to a tensor type of the second colorant according to the contracting and the confusion of first with a law experience of the medical scient, the exercising scaling places under conditions effective to allow hybridizations of the disputational case that the superprinciple with the suscious and

- 10 observing a detectable change brought about by hybridization of the oilgocurisotides on the assoparticles with the nucleic acid.
  - The method of Claims 269 wherein the contacting confidence include freezing and thawing.
  - The method of Claim 269 wherein the controling conditions include heating
- 272. The method of Claim 269 wherein the detectable change is observed on a 20 solid surface.
  - 273. The method of Claims 269 wherein the detectable change is a color change observable with the naked sys.
- 25 274. The method of Claim 273 wherein the color change is observed on a solid auriton.
  - 275. The method of Claim 269 wherein the nanopurticles are tretal

PCT/R80L/01396

#### nanoparticles or semiconductor nanoparticles

- 276. The method of Claim 269 whorein the nanoparticles are gold nanoparticles.
- 277. The method of Claims 269 wherein the oligenucleorides attached to the nanoparticities are listeded an their ends not attached to the nanoparticities with molecules that produce a detectable change upon hybridization of the oligenucleotides on the nanoparticities with the nucleic atol.
- 278. The method of Claims 277 wherein the maneparticles are mostilic or semiconductor maneparticles and the oligopout colides attached to the maneparticles are labeled with fluorescent molecules.
- 279. The method of Claim 269 wherein:
- the nucleic acid has a third portion located between the first and second partions, and the sequences of the oligonucleotides on the nanoparticles do not include sequences complementary to this third portion of the nucleic acid; and
- the nucleic acid is further contacted with a filler oligonucleotide having a 20 sequence complamentary to this third portion of the nucleic acid, the contacting taking place under conditions effective to allow bytoricization of the filter oligonucleotide with the nucleic acid.
  - 280. The method of Claim 269 wherein the nucleic said is viral RNA or DNA.
  - 281. The method of Cisim 269 wherein the nucleic sold is a gene associated with a disease.

WO 03/51/65

PC-T/USDI/R1199

- 282. The method of Claim 269 wherein the nucleic sold is a bacterial DNA.
- 283. The method of Claim 269 wherein the quelolo acid is a fungal DNA.
- 5 284. The method of Claims 269 wherein the nucleic sold is a synthetic DNA, a synthetic RNA, a structurally-modified natural or synthetic RNA, or a structurally-modified natural or synthetic RNA.
- 285. The method of Claim 269 wherein the nucleic solid is from a biological 10 source.
  - 286. The method of Claims 269 wherein the matche acid is a product of a polymerase chain reaction amplification.
- 15 287. The method of Claims 269 wherein the nooleic sold is contacted with the first and second types of cortiugates simultaneously.
- 288. The method of Chim 269 winterin the models said is contacted and
  hydridized with the alignmeteotides on the renoparticles of first type of conjugates

  20 before being contacted with the second type of conjugates.
  - 289. The method of Claim 288 wherein the first type of conjugates is attached to a substrate.
- 25 290. The method of Claim 269 whorein the michele sold is double-stranded and hybridization with the oligomodeotidas on the nanoparticles results in the production of a triple-stranded complex.

WO 91/51665

PCT/CSRL/II 190

- . 291. A method of detection a sucleic sold having at least two portions comprising:
- providing a type of nanoparticles seconding to any one of Claims 243-252 having recognition digeometrolides statched thereto, the recognition oligeometrolides on such manaparticle comprising a requester complementary to the sequence of at least two portions of the subtles add;
- contacting the nucleic acid and the numeratives under conditions effective to allow hybridization of the oliganucleotides on the numeratives with the two or more partices of the nucleic acid; and
- 10 observing a detectable change brought about by hybridization of the oligonucleotides on the ranoparticles with the zucleic acid.
- 292. A method of detecting multic solid having at least two portions
- to containing the secular soft with a last me to type of amountaining contributing to an official model 200 billing recognition eligenmentates attented themes, the recognition eligenmentative as there are for first type of assopration configurations in a secular configuration of the exquance of the matter south exist, the temperature comprision coligenmentation on the secural type of assopration comprision coligenmentation as second position of the recognition comprision comprision configuration and the second position of the control recognition comprision and the control of the control recognition comprision and the control of the recognition control of the recogni
- observing a detectable change brought about by hybridization of the econgnition oligorusclostides on the nanoparticles with the nucleic acid.

  25
  - 293. The method of Claim 292 wherein the contecting conditions include freezing and flawing.

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- 254. The method of Claim 292 wherein the contacting conditions include heating.
- 295. The method of Claim 292 wherein the detectable change is observed on a 5 —solid surface.
  - 295. The method of Claim 292 wherein the detectable change is a color change observable with the naked eye.
- 0 297. The method of Claim 296 wherein the color change is observed on a solid
- 298. The method of Claim 292 wherein the nanoparticles are metal nanoparticles or semiconfuctor ramoparticles.
  - 299. The method of Claim 298 wherein the panoparticles are made of gold.
- 500: The method of Claim 292 wherein the recognition oligenecheolides attached to the recognition and interior most attached to the recognitions are labeled on their code not attached to the recognitions with molecules that produces a detectable change upon hybridization of the obsparratements on the management of the department of the management of the management of the department of the management of th
- 301. The method of Claim 300 wherein the nanoparticles are metallic or numiconductor nanoparticles and the oligonucleotides attached to the nanoparticles are labeled with fluoresteet molecules.
  - The atethod of Claim 292 wherein:
     the motion acid has a third pertion located between the first and second

WU 01/51665

PCT/USHIAH 190

portions, and the sequences of the oligonucleosides on the manoparticles do not include sequences complementary to this third portion of the nucleic acid; and

the moletic acid is further consucted with a filler oligonuclostoide having a sequence complementary to this third portion of the moletic acid, the contenting taking place under conditions effective to allow hybridination of the filter oligonuclostide with the multicle acid.

- 303. The method of Claim 292 wherein the models sold is viral RNA or DNA.
- 10 304 The method of Claim 292 wherein the nucleic sold is a gene associated with a disease.
- 305. The method of Claim 292 wherein the nucleic acid is a bacterial DNA.
- 15 306. The method of Claim 292 wherein the nucleic acid is a fungel DNA.
- 307. The method of Claims 292 wherein the modeic acid is a synthetic DNA, a synthetic RNA, a structurally-modified natural or synthetic RNA, or a structurally-modified natural or synthetic DNA.
- 308. The nothed of Claim 292 wherein the nucleic uoid is from a biological
- 309. The method of Chairn 292 wherein the nucleic sold is a product of a polymerase chain reaction amplification.
  - 310. The method of Claim 292 wherein the nucleic acid is contacted with the first and second types of panoperticles simultaneously.

PCT/USHL01150

- 311. The method of Claim 292 wherein the moleic acid is contacted and hybridized with the oligonucleotides on the first type of nanoparticles before being contacted with the second type of assoparticles.
- 312. The method of Climit 311 wherein the first type of nuneparticles is attached to a substrate.
- 313. The method of Claim 292 wherein the nucleic acid is double-stranded and 10 hybridization with the oligonucleotides on the campanticles results in the production of a triple-stranded complex.
  - 314. A method of detecting a mucleto axid having at least two perticus comprising:
- providing a type of nanoproficies according to any one of Claims 253-265

  having recognition of ligorous/cordina statedard therety, the recognition oligorous/cordina statedard therety, the recognition oligorous/cordina as each nanopratitic comprising a popurace complementary to the sequence of at least two portions of the models acid;
- contacting the modele sold and the nanoparticles under conditions

  20 effective to allow hybridization of the recognition of generaleotides on the manoparticles
  with the two or more portions of the metric acid; and
- observing a detectable change brought about by hybridization of the recognition of groundestides on the nanoparticles with the nucleic acid.
- 315. A method of detecting nucleic acid having at least two portions comprising:

contacting the nucleic acid with at least two types of nanoparticles according to any one of Claims 253-263 having recognition oligomacleotides attached

PCT/USHI/RI 190

thereto, the recognition oligoconteorides on the first type of annoparticles comparing a sequence complementary to a first periods of the sequence of the matries said. the recognition oligopathecedure one become fully per damagnatization comprising a sequence complementary to a second portion of the requester of the nucleic soid, the contenting 5 taking place under conditions effective to allow hybridisation of the recognition obligantupolities for the numeratical wide mutation skills of the property of international control property of the nucleic skills and disputational to one numeratical wide mutation skills are

observing a detectable change brought about by hybridization of the recognition of genucleotides on the nanoparticles with the nucleic acid.

- 316. The method of Claim 315 wherein the contacting conditions include freezing and thawing.
- 317. The method of Claims 315 wherein the contacting conditions include
  - 318. The method of Claim 315 wherein the detectable change is observed on a solid surface.
- 319. The method of Claim 315 wherein the detectable change is a color change 20 observable with the naked eye.
- 320. The method of Claim 319 wherein the color change is observed on a solid
- 3 321. The method of Claim 315 wherein the pamoparticles are smotal managarificies or senticonductor nanoparticles.
  - 322. The method of Claim 321 wherein the nanoparticles are made of gold.

PCT/RSDI/01190

- 323. The method of Claims 315 wherean the recognition ofignosedootides attaibed to the managerificies are labeled on their ends not attaibed to the managerificies with molecules that produce a detectable change upon byto-initiation of the recognition of ignorecipation with the nucleic said.
- 324. The method of Claim 323 wherein the assoparticles are metallic or semiconfutiver precipitates and the recognition oligonucleosides attached to the nanoparticles are labeled with flaversecent molecules.
  - 325. The method of Claim 315 wherein:

the nucleic acid has a third portion located between the first and second positions, and the sequences of the oligonucleotides on the nanoparticles do not include sequences complementary to this third portion of the nucleic sold; and

the nucleic acid is further contented with a filler oligonatestatide having a sequence complementary to this thard pursons of the nucleic acid, the connecting taking places under conditions effective to allow hybridization of the filter oligonacteoide with the nucleic acid.

- 326. The method of Chien 315 wherein the nucleic acid is viral RNA or DNA.
- 327. The method of Claim 315 wherein the nucleic acid is a gone associated with a disease.
- 328. The method of Claim 315 wherein the nucleic sold is a besterial DNA.
  - 329. The method of Claim 315 wherein the nucleic sold is a fungal DNA.

WO 03/51045

PCT/CSUL/01190

- 330. The method of Claim 315 wherein the motitic acid is a symbotic DNA, a symbotic RNA, a symbotic RNA, a structurally-modified natural or symbotic RNA, or a structurally-modified natural or synthetic DNA.
- 331. The method of Claim 315 wherein the nucleic acid is from a biological source.
  - 332. The motiod of Claim 315 wherein the modele acid is a product of a polymerate chain reaction amplification.
  - 333. The method of Claim 315 wherein the nucleic sold is contacted with the first and second types of metoperticles simultaneously.
- 334. The antipol of Claim 315 wherein the autilities odd is contacted and 15 hybridized with the recognition oligomericotides on the first type of nanoparticles before being contacted with the second type of nanoparticles.
- 335. The method of Claim 334 wherein the first type of nanoparticles is attached to a substrate
- 336 The tortiod of Claim 315 wherein the nucleic acid is double-strended and hybridization with the oligoeucleotides on the nanoparticles results in the production of a triple-strended complex.
- 25 337. A method of detecting a nucleic acid having at least two portions commission:
  - (a) contacting the nucleic acid with a substate having oligonucleotides attached thereto, the oligonucleotides having a sequence complementary to a first portion

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of the sequence of said nucleic axid, the contacting taking place under conditions effective to allow hybridusation of the oligonauteotides on the substrate with said nucleic axid:

(b) controlling and mustele sold bound to the nobistions with a first type of immorphical-colligorationide conjugates according to say one of Chim 237-340, at least one of the types of climpattodes attained to the museparticle of the conjugates lawing a sequence complementary to a second popular of the sequence of and seculid modify, the confined confident feature by the configuration of the objective to the Chimical Seculidaries of the objectives with said auxiliar acids.

### (c) observing a detectable change.

## 338. The method of Claim 337 further comprising:

(8) containing the fact type of anapprecisive-dispursationative conclusions to bound to the substant with a second type of anapprecisive-dispursationative conjusters asserting to say one of Cultura (2072-86), at least one of the type of dispursationation for the resource of the type of dispursationation of the resource type of the supersations of the resource type of the supersations of the resource of east of the types of dispursationation attained to the commence of the order for type of of dispursationation attained to the commence of the order type of order to be contenting tables placed and confident to a flow types of dispulsationation of the compensation of the contenting tables placed and confident to the order types of complexity and the first set of contenting tables placed to the compensation of the client type of complexity and the first set of contenting tables placed to the compensation of the client type of complexity and the contenting tables placed to the compensation of the client type of complexity and the contenting tables placed to the compensation of the client type of complexity and the contenting tables are the contenting tables placed to the compensation of the client type of complexity and the contenting tables placed to the compensation of the client type of complexity and the contenting tables are the contenting tables the content tables are the contenting tables are the contenting tables are

(e) observing the detectable change.

339. The method of Claim 338 wherein at least one of the types of 25 oligamentoolides on the nanoparticles of the first type of conjugates has a sequence complementary to the sequence of at least one of the types of oligamenteeddes on the sympacticles of the second type of conjugates and the method further comprises:

(f) contecting the second type of conjugates bound to the substrate with

PCT/USHL/01198

the first type of conjugates, the consecting taking place under conditions effective to allow hybridization of the oligonucleoxides on the nanopurticles of the first and second types of conjugates; and

(g) observing the detectable change.

340. The method of China 339 wherein step (d) or steps (d) and (f) are repeated one or more times and the detectable change is observed.

# The method of Claim 337 further comprising: (d) providing a type of binding oligonuclootides having a sequence

- (a) providing a type of olinting ongeniciones having a sequino comprising at least two portions, the first portion being complementary to at least one of the types of oligonucleotides attached to the namoparticles of the first type of conjugates;
- (e) contacting the binding oligonarizations with the first type of conjugates board to the subsette, the contacting taking place under conditions effective to allow hybeidization of the binding oligonarizations with the oligonucleotides on the supportations of the first type of conjugates;
- (f) providing a account type of manoparticle-alignmentenide conjugates according to any one of Chimna 237-240, as least one of the types of olignmentenide attached to the amonparticles of the account type of conjugates having a sequence complementary to the account of the acquence of the binding olignmentenides;
  - (g) contacting the binding oligonust entities bound to the substrate with the second type of conjugates, the contacting taking place water conditions effective to allow hybridisation of the oligonus/contides attached to the nanoparticles of the second type of conjugates with the binding oligonus/contact, and

(h) observing the detectable change.

342. The method of Claim 341 farther comprising:

(i) contacting the second type of conjugates bound to the substrate with the

PCDUSHIJI 194

binding eligensuloctides, the contenting taking place under conditions effective to allow hybridization of the binding eligensuloctides with the eligensulectides on the nanoparticles of the second type of conjugator;

(i) contacting the binding oligonucleoides bound to the substrate with the 5 first type of conjugates, the contacting takin piece under conditions effective to allow hybridization of the oligonucleoides on the nanoparticles of the first type of conjugates with the binding oligonucleoides; and

(k) observing the detectable clumps.

- 10 343. The method of Claim 342 wherein stops (e) and (g) or steps (e), (g), (i) and (j) are repeated ont or more fames, and the detectable change is observed.
  - 344. The method of Chaim 337 wherein the substrate is a transparent substrate or an opaque white substrate.
  - 345. The method of Claim 344 wherein the detectable change is the formation of dark seess on the substrate.
- 366. The method of Claum 337 wherein the nanoparticles of the conjugates are 20 metal ranoparticles or semiconductor nanoparticles.
  - 347. The method of Claim 346 wherein the nanoparticles of the conjugates are made of gold or silver.
  - 5 348. The method of Claim 337 wherein the substate has a plurality of types of oligonaulocoides attached to it in an array to allow for the detection of multiple portions of a single nucleic acid, the detection of multiple different nucleic acids, or both.

PCT/US0L/01390

- 349. The method of Claim 337 wherein the substrate is contacted with silver stain to produce the detectable change.
- 350. The method of Claim 348 wherein the substrate is contacted with aliver 5 stein to produce the detectable change.
  - The method of Claims 337 whosoin the detrotable change is observed with an optical seasoner.
- 10 352. The method of Claim 351 wherein the device is a flatbed scanner.
- 353. The method of Claim 351 wherein the assence is linked to a computer loaded with software capable of calculating groysate measurements, and the proyection measurements are calculated to provide a quantitative measurement are calculated to provide a quantitative measurement of nucleo acid detented.
- 354. The method of Claim 337 wherein the oligosucleotides attached to the substates are located between two electrodes, the nanoparticles of the conjugates are made of a moterial which is a conquestor of electricity, and the detectable change is a claimer in confunctivity.
- The method of Claim 354 wherein the electrodes are made of gold, and the managarticles are made of gold.
- 35. The method of Claim 334 wherein the substrate is contacted with silver stain to produce the change in conductivity.
  - 357. The method of Claim 348 wherein each of the plurality of

WO 91/51/65

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oligonationides statched to the substrate in the array is located between two electrodes, the nanoparticles are made of a material which is a conductor of electricity, and the detectable change is a change in conductivity.

- 5 358. The method of Claim 357 wherein the electrodes are made of gold, and the nanocarticles are made of gold.
  - 359. The method of Claim 357 wherein the substrate is contacted with silver stain to produce the classing in conductivity.
- 360. A method of detecting a mucleic acid having at least two portions comprising:
- (a) contacting the metales axid with a robotant having dignomacleosides allocated thereto, the eligensteeledes having a requere complementary to a first period of the sequence of said mustles said, the contacting skinley place under conditions effective to allow hybridization of the oligonucleosides on the substrate with said nucleic
- (b) oculating said motion field bound to the substants with a first type of emporations according to any one of Cipient 24/200 busing one or more types of 20 more produced authority of the control of the production of comprisions standard abstract, a listent cost of the type of recognition of comprisions are supersectively to a second position of the sequence comprising a sequence completements you is accord position of the sequence of and proteins said, the entereding tablest place unforce confinementations to allow hybridizations of the disposalisations on the support of the sequence of the disposalisation of t

(c) observing a detectable obasge.

36). The mothed of Claim 360 further comprising:
(d) contenting the first type of amopacticles bound to the substrate with a

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second type of nanoparticles exceeding to say one of Chima 243-259 having recognition object-incides unbond thereou, at least one of the system of recognition alignmenterists are on the second type of manageristic comparings a recognition on the second type of manageristic comparings at requirementary to this surjection of one of the types of object-incides on the first type of manageristics, this police of the object of the second type of the se

(e) observing the detectable change.

362. The ratched of Claim 360 wherein at least one of the types of recognition o digensclenides on the fart type of amorphicide has a sequence complementary to the sequence of at least one of the types of oligonalestides on the second type of manaparticle and the seatched father comprises:

(f) contacting the second type of nanoparticlos bound to the substants with the first type of nanoparticles, the contacting taking place under conditions effective to 15 allow hybridization of the oligosuclooides on the first and second types of nanoparticles, and

(g) observing the detectable charge.

363. The method of Claim 362 wherein step (d) or steps (d) and (i) are repeated 20 one or more times and the detectable clamps is observed.

364. The method of Claim 360 further comprising:

(d) providing a type of kinding oligonucleotides having a sequence
comprising at least two portions, the first portion being complementary to as least one of
the types of oligonucleotides on the first type of nanoparticles;

(e) contenting the binding oligonechectides with the first type of nameparticles bound to the substrate, the contacting taking place water conditions effective to allow hybridization of the binding oligonucleotides with the oligonucleotides.

WU 01/51665

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on the first type of nanoparticles;

(f) providing a second type of immognitizate according to any one of Claims 24-250 barries prougation oligomedestria strated thereto, at least one of to types of recognition oligometerisms under second type of amorparticle complaints a 5 sequence complementary to the second portion of the tequence of the binding obligometeristics;

(g) contesting the binding objectuation/des bound to the substrate with the second type of nanoparticles, the contesting taking place under conditions effective to allow hybridization of the objectual contesting taking place under conditions effective to allow hybridization of the objectual contesting the second type of nanoparticles with the binding objectuation/destructions.

(h) observing the detectable change.

# 365. The method of Claim 364 further comprising:

(i) contacting the second type of mapparticles bound to the substrate with
the binding oligorescleotides, the contacting taking place under conditions effective to
allow hybridization of the binding oligonuclootides with the oligonuclootides on the

second type of nanoparticles;
(i) contacting the binding oligomatestides bound to the substrate with the
first type of nanoparticles, the contacting taking place under conditions effective to allow
their distriction of the oligomatestates on the first type of nanoparticles with the binding

oligometeotides; and (k) observing the detectable change.

366. The method of Claim 365 wherein steps (e) and (g) or steps (e), (g), (i) and
25 (j) are repeated one or more times, and the detectable change is observed.

367. The method of Claim 360 wherein the substrate is a transparent substrate or an opaque white substrate.

WO 01/51/65

PC37/0500/01390

- 368. The method of Chairm 367 wherein the detectable change is the formation of dark areas on the substrate.
- 369. The method of Claim 360 wherein the nanoparticles are metal managerticles or semiconductor number tiles.
- 370. The method of Claim 369 wherein the nanoparticles are made of gold or either.
- 371. The method of Claim 360 wherein the substrate has a plurality of types of oligoneticotides satisfated to it in an array to allow for the detection of multiple portions of a single nucleic acid, the detection of multiple different nucleic acids, or both.
- 15 372. The method of Claim 360 wherein the substrate is contacted with aliver stain to produce the describble change.
- 373. The method of Claim 371 wherein the substrate is contacted with allver stale to produce the detectable change.
- 375. The method of Claim 360 wherein the detectable change is observed with an optical scenner.
  - 376. The method of Claim 375 wherein the device is a flatbed season.
  - 377. The method of Claims 375 wherein the scenuer is lighted to a computer loaded with software capable of calculating properly measurements, and the properly measurements are calculated, to provide a quantitative measure of the amount of nucleic

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#### acid detected.

- 378. The method of Claim 366 wherein the oligonucleotides attached to the substrate are located between two electrodes, the ranoparticles are made of a material 5 which is a conductor of electricity, and the detectable change is a change in constructivity.
  - The method of Claim 378 wherein the electrodes are made of gold, and the nanoparticles are made of gold.
- 10 380. The control of Chairs 378 wherein the substrate is contacted with silver state to produce the charge in confactivity.
- 381. The method of Claim 371 wherein each of the plausility of oligonucleotides attended to the addetune in the army is learned between two electrodes.

  15 the macaparticles are made of a material which is a conductor of electricity, and the determine durings is a change in confidentivity.
  - 382. The method of Claim 381 wherein the electrodes are made of gold, and the memoperticles are made of gold.
  - 383. The method of Claim 381 wherein the substrate is contacted with silver stain to produce the change in conductivity.
- 334. A method of detecting a nucleic solid having at least two portions 25 comprising.
  - (a) contesting the nucleio oxid with a substrate having oligonucleosides attached thereos, the oligonucleosides having a sequence complementary to a first portion of the sequence of said nuclein seid, the contacting taking place under conditions

WO 03/51/15

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effective to allow hybridization of the oligonucleotides on the substrate with said nucleic

- (c) constraint partial models into board to the solutionare with a fact type of annoquerious according to say one of Calcium 273-263 having one or more types of 5 recognition oligometricotions attacked fittems, at least one of the types of renognition oligometricotical completing and the completion of the properties of the properties
  - (c) observing a detectable change.

#### 385. The method of Claim 384 further comprising:

(d) containing the first type of incorporation bound to the substance with a second by one of managemental as recording to an office man 233-256/mg; recognition 15 objects extracted theretals, at least one of the types of recognition objects extracted theretals, a least one of the types of recognition objects extracted on the second type of managementals securities) a sequence of complementary to the sequence of complemental to the part of objects their terms to the first the order integrations, the consistency to the part of objects the ordering the objects to the first type of immediated, the consistency to the part of objects the ordering the objects to the ordering the objects the ordering the ordering the ordering the objects the ordering the order

### (e) observing the detectable change.

386. The method of Claim 385 wherein at least one of the types of recognition oligonuclosities on the first type of intooperiolise comprises a sequence complementary the sequence of it least one of the types of oligonuclosities on the second type of prepositions and the method father comparise:

(f) contacting the second type of assopurations bound to the substrate with the first type of assopurations, the constacting taking place under conditions effective to allow hybridization of the oligonucleotides on the first and second types of remognations;

WO 01/51/45

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(g) observing the detectable change.

387. The method of Claim 386 wherein step (d) or steps (d) and (f) are repeated 5 one or more times and the detectable change is observed.

## 388. The method of Claim 384 further comprising:

(d) providing a type of binding oligonucleotides having a sequence comprising at least two portions, the first portion being complementary to at least one of 10 the types of oligonucleotides on the first type of nanoparticles;

(c) contacting the binding offgornolectides with the first type of nanoparticles bound to the substrate, the contecting taking piece under conditions effective to allow hybridization of the binding oligonucleotides with the oligonucleotides on the first type of nanoparticles,

(f) providing a second type of nanoparticles according to any one of Chiese 253-263having recognition oligonacleotides attached thereto, at least one of the types of recognition oligonucleotides on the second type of nanoparticles comprising a sequence complementary to the second portion of the sequence of the binding oligonusteorides;

(g) contacting the binding of grant leatides bound to the substrate with the second type of nanoperticles, the contacting taking place under conditions effective to allow hybridization of the oligonosleotides on the second type of nanoparticles with the binding oligonucleotides; and

(b) observing the detectable change.

# 389. The method of Claim 388 further comprising:

(i) contacting the second type of nanoparticles bound to the substrate with the binding oligonucleotides, the contacting taking place under conditions effective to

PCT/USRI/U1390

- allow hybridization of the binding oligonucleotides with the oligonucleotides on the second type of nanoparticles;
- (i) contacting the binding oligonucleotides bound to the substrate with the first type of nanoparticles, the contacting taking place under conditions affective to allow hybridization of the oligonucleotides on the first type of nanoparticles with the binding eligonucleotides, and
  - (k) observing the detectable change.
- 390. The method of Claim 389 wherein steps (e) and (g) or steps (e), (g), (i) and to (j) are repeated one or more times, and the detectable change is observed.
  - The nothed of Claim 384 wherein the substrate is a transparent substrate or an opaque white substrate.
- 15 392. The suchod of Claim 391 wherein the detectable change is the formation of dark areas on the substrate.
- 393. The method of Claims 384 whorein the nanoparticles are metal nanoparticles or semiconductor nanoparticles.
- The method of Claim 393 wherein the nanoparticles are made of gold or silver.
- 305. The method of Claim 384 wherein the substante has a plurality of types of oligonucleotides attached to it in an array to allow for the detection of multiple portions of a single nucleic acid, the detection of multiple different nucleic acids, or both.
  - 396. The method of Claim 384 wherein the substrate is contacted with silver

PCT/USH/01196

#### stain to produce the detectable change

- 397. The method of Chaim 395 wherein the substrate is contacted with sliver stain to produce the descetable change.
- 398. The method of Claim 384 wherein the detectable change is observed with an optical actuater
- 399. The method of Claim 398 wherein the device is a flathed scanner.
- 400. The method of Claim 396 wherein the scanner is linked to a computer loaded with software capable of calculating greyscale measurements, and the greyscale measurements are calculated to provide a quantitative measure of the amount of nucleic acid detected.
- 401. The method of Claim 384 wherein the oligonucleotides attached to the substrate are located between two electrodes, the nanoparticles are made of a miserial which is a conductor of electricity, and the detectable change in a change in conductivity.
- 402. The method of Claim 401 wherein the electrodes are smade of gold, and the numeraticles are made of gold.
- 403. The merical of Claim 401 wherein the substrate is contacted with silver stain to produce the change in conductivity.
- 404. The method of Claim 397 wherein each of the plurality of oligonucteotides otherhol to the substante in the array is located between two electrodes, the manaparticles are made of a material which is a conductor of electricity, and the

WO 01/51/65

ectional no

#### detectable change is a change in conductivity.

- 405. The method of Claim 404 wherein the electrodes are made of gold, and the manoparticles are made of gold.
- 405. The method of Claism 404 wherein the substrate is contracted with silver state to produce the charge in conductivity.
- 407. A method of detecting a nucleic acid having at least two portions
   10 comprising:

(4) centering the motifies and with a substate having observables the state of the control of the sequence of the control of the control of the sequence of the control of the contr

(b) contacting and materials and bound to the sharmar with a first type of mecognitics, the grant and such as some state and the sharmar with a first type of a material whole consider identicity, the meroparticism being one or more types of oligomoticetoise standard theretoe, at issue of the types of dispressionables beings a sequence complomatory to a smooth of the types of dispressionables beings as expenses exceptionablery to a store position of the response of red materials and the controlling taking being water confidence of the controlling taking being water confidence and the controlling taking being water confidence and the controlling taking being and the confidence and the controlling taking being and the confidence and the confidence and the controlling taking taking the controlling taking the controlling taking the controlling taking taking the controlling taking ta

#### (c) detecting a change in conductivity.

5 409. The method of Claim 407 wherein the substrate has a phralify of pairs of electedes located on it in an array to allow for the detection of antihiple portions of a single nucleic acid, the detection of nutlitiple different nucleic acids, to both, each of the pairs of electrosics having a type of objectational statched to the substrate horseas.

WO 01/51/655

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then

- 409. The method of Claim 407 wherein the nanoparticles are made of metal.
- 5 410. The method of Claim 407 wherein the temperaticles are made of gold or silver.
  - 411. The method of Claim 407 wherein the substrate is contacted with silver stain to produce the charge in conductivity.
    - 4)2. The method of Claim 407 further comprising:

(d) contexting the first type of annoparticles board to the otherston with a second type of annoparticles the sampesticles being used of a material which on conduct satesticely be annoparticles being used (particularists annotated therein, or least 15 one of the types of ofiguranticelists on the second type of annoparticles completing a segmence complementary to the sequence of an of at thy peri of objectively to the sequence of an of at thy peri of objections on the first type of encoparticles, the containing taking pioce under conditions effective to all the period objective of the complete of the conditions of

- (e) detecting the change in conductivity.
- 413. The worked of Claim 412 wherein at least one of the types of oligonuclosities on the first type of canoparticles has a sequence complementary to the conjument of all least our of the types of oligonuclosities on the second type of manaparticles and the method firstfar comprises:
- (f) contooing the accend type of nanoparticles board to the substrate with the first type of nanoparticles, the contacting taking place under conditions effective to allow hybridization of the oligonucleotides on the first and accord types of nanoparticles;

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#### (g) detecting the change in conductivity.

- 414. The method of Claim 413 wherein 20p (d) or steps (d) and (f) are repeated one or more times and the change in conductivity is detected.
  - 415 The method of Claim 407 further comprising:
- (d) controling the first type of suspendid sound to the substruct with a suggressive behavior all spendid fromts, the supposed to the size all spendid sounds as the suppose probe below and set a meetal which non-contact electricity, at learn one of the type of disquarkations on the suggester pulse soundings a sequence complementary to the sequence of one of the type of disquarkations are the suppose of one of the type of disquarkations are the suppose of the sequence of one of the type of disquarkations are the supposed of the supposed contacting tabling times cannot contacting challenges and the contacting challenges are their contactions differed to a finite behavior of the supposed contacting challenges are the contacting challenges and the supposed contacting challenges are the supposed to the supposed contacting challenges are the supposed to the supposed contacting challenges are the supposed contacting challenges are the supposed to the supposed contacting the supposed contacting the supposed contacting challenges are the supposed contacting challenges are the supposed contacting the supposed contacting challenges are the supposed contacting challenges are the supposed contacting the supposed contacting challenges are the supposed challenges are the supposed
  - (e) and detecting the change in confuctivity.
  - 416. A mosthod of detecting nucleic acid having as least two portions competiting:
- (c) contacting a surcleix sold with a substrate having oligonoschoolides and another than the contract of the contract of the contract of the object-motorists being a sequence complementary to a first provision of the sequence of said moticies soid, the contaming taking piles under conditions effective to allow hybridications of hor illegonate-tolines on the reherment with offended soids of the piles of the contraction of the contraction of the contraction of the contraction of the piles of the contraction of the contraction of the contraction of the contraction of the piles of the contraction of the contraction of the contraction of the piles of the contraction of the contraction of the contraction of the piles of the contraction of the contraction of the contraction of the piles of the contraction of the contraction of the contraction of the piles of the contraction of the contraction of the contraction of the piles of the contraction of the contraction of the contraction of the piles of the contraction of the contraction of the contraction of the piles of the contraction of the contraction of the contraction of the piles of the contraction of the contraction of the contraction of the piles of the contraction of the contraction of the contraction of the piles of the contraction of the contraction of the contraction of the piles of the contraction of the
- (b) constituting and machine and bound to the solution with an aggregate probe having oligomethrodists attended thesters, at least one of the types of oligomethrodists on the aggregate probe comprising a sequence complementary to the sequence of a second potion of said anachie tool, the meaporticles of the aggregate probe being made of a martial beloak our coduct electricity, the constating stimule.

WO 01/51/65

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under conditions effective to allow hybridization of the oligonucleotides on the aggregate probe with the nucleic acid; and

(c) detecting a change in conductivity.

- 417. A method of detecting a nucleic acid wherein the method is performed on a substrate, the method comprising detecting the presence, quantity, or both, of the nucleic acid with an optical scanner.
  - 418. The method of Claim 417 wherein the device is a flathed scanner.
  - 419. The method of Claim 417 wherein the acaroner is linked to a computer loaded with software capable of calculating groyonale measurements, and the preparable measurements are calculated, to provide a quantistive measure of the amount of meclaic add detected.
  - 420. The atchool of Claim 417 wherein the scenner is linked to a computer loaded with software capable of providing an image of the substrate, and a qualitative determination.
- of the prosence of the speleje acid, the quantity of the nucleic acid, or both, as made.
- A kit comprising a container holding nanoparticle-oligonucleoride conjugates according to any one of Claime 237-242.
- 422. A kit comprising a container holding nanoparticles according to any one
  25 of Ctains 243-265.
  - 423. A kit comprising a substrate having attached thereto at least one pair of electrodes with oligomorelectides attached to the substrate between the electrodes.

PCT/USBL/01291

424. The bit of Claim 423 wherein the substrate has a plurality of pairs of electrodes attached to it in an array, to allow for the detection of multiple portions of a single nucleic sold, the detection of multiple different models acids, or both.

#### 425. A method of nanofabrication comprising

providing at least one type of linking oligonucleotide having a selected sequence, the sequence of each type of linking oligonucleotide having at least two positions.

- providing one or more types of nanoparticle-digencelestide conjugates according to any one of Claims 237-247, the oligonucteotides statched to the nanoparticles of each of the types of conjugates having a sequence complementary to the sequence of a portion of a likiting oligonucteotide, and
- containing the lishing ofigoracioniste and conjugates under conditions

  15 effective to allow hybridization of the oligonoutsolides attached to the annoparations of
  the conjugates to the lishing oftigonoutsolides to that is desired annomatestal or
  mensatrotorie is formed wherein the nanoparticles of the conjugates are held together, by
  oligonoutice/disconstrum.

#### Q 426. A method of camofabrication comprising

providing at least one type of linking oligonucleotide having a selected sequence, the sequence of each type of linking obgennelectide having at least two portions;

providing one or more types of nanoparticles according to any one of 25 Chains 243-265, the recognition obligame/tentides on each of the types of asseparables competing a sequence complementary to the sequence of a portion of a linking obligame/textide; and

contacting the linking oligomaticates and nanoparticles under conditions

PC DUSTUM 191

effective to allow hybridization of the obgonucleotides on the nanoparticles to the limiting obgonucleorides so that a desired manomaterial or nanoparticles is formed wherein the nanoparticles are held together by objectuacycloide connectors.

### 427. A method of nanofabrication comprising:

providing at least two types of nameparticle-oligenucleoside conjugates according to any one of Ctaims 237-242,

the oligonic-holides attached to the nanoparticles of the first type of conjugates having a sequence complementary to that of the oligonic-holides attached to the nanoparticles of the second type of conjugates;

the oligonaclootides attached to the nanoparticles of the second type of conjugates having a sequence complementary to that of the oligonacisotides attached to the nanoparticles of the first type of conjugates, and

contacting the first and second types of conjugates under conditions

15 effective to allow hybridization of the oligonucleotides on the nanopusticles of the conjugates to each other so that a desired nanomaterial or nanopurocure is formed.

#### 428. A method of panofabrication comprising:

providing at least two types of nanoparticles according to any one of 20 Chims 243-265.

the recognition oligonucleotides on the first type of numoparticles comprising a sequence complementary to that of the oligonucleotides on the second of the succounticles;

the recognition ofigurescientists on the second type of manupleritides
25 comprising a sequence complementary to that of the oligonocleotides on the first type of
neospecificus; and

contacting the first and second types of nanoparticles under combitions effective to allow hybridization of the objectuelectides on the nanoparticles to each other

WO 93/51665

PCT/USBIAD 191

so that a desired nanomaterial or nanostructure is formed.

- 429. Nanomatorials or nanosinustures composed of nanoparticlealignucleotide conjugates according to any one of Claims 237-242, the nanoparticles being held together by oligomaterated connectors.
  - 450. Neutoratorisis or nanostructures composed of nanoparticles according to say one of Claims 243-265, the nanoparticles being hold together by eligonactorisis commetters.
  - 431. A method of separating a selected modele cold having at least two portions from other nucleic ucids, the method comprising:
- providing two or more types of nanoparticle-oligoracleotide conjugues occording to any one of Claims 237-242, the oligonucleoides atteched to the 15 nanoparticles of each of the types of conjugates having a sequence complementary to the sequence of one of the perions of the selected nutricle soid; and
- contacting the medicin exists and conjugates under conditions effective to allow hybridization of the oligonacleoxides on the nanoparticles of the conjugates with the selected nutcleic acid so that the conjugates hybridized to the selected nutcleic neid aggregate and procipitate.
  - 432. A mothed of apparating a solected nuclair acid having at least two portions from other nucleic solds, the method comprising:
- providing two or more types of unreparticles according to any one of 25 Claims 243-255, the oligonacteolides on each of the types of nanoparticles having a sequence complementary to the sequence of one of the portions of the selected nucleis acid; and

contacting the nucleic acids and renoperticles under conditions effective

PCT/USOLAN 1911

to allow hybridization of the oligomaclostides on the annoparticles with the selected nucleic acid so that the annoparticles bybridized to the solected nucleic acid aggregate and precipitate.

- 433. Nanoparticle-oligonacieotide conjugates which are nanoparticles having obigonucleotides attached to them, the oligonucleotides having a covalently bound cyclic disulfide functional group that can bind to the nanoparticles.
- 434. Nanoparticle oligonaccionide conjugates which are nanoparticles having 10 oligonacciolides attacted to them, the oligonaccionides having a covalently bound polythiol functional group that can bind to the nanoparticles.
- 415. Menoputific-indigenentendes conjugates which are manoperticles having oligonoutcodien standards in them, the oligonoutcodien standards in them, the oligonoutcodien standards are consultantly bound cyclic 15 deselfale facetional group that can bind to the emoparation, at least some of the oligonoutcoids having a sequence complementary to at least one position of the sequence of an archivele sold or mosther oligonoutcoids.
- 456. Nanoparticio-digenentinotde conjugette which are assorprinted having 20 dispossationides statede to them, the objectoritoide having an operation population and the proposition are propositional proxy late can be allow the suspensition, at least some of the objectoritoide having a sequence complementary to at least one portion of the segment of a randotte door number objectoritoide.
- 25 437. The conjugates of elsims 435 or 436 wherein the oligosucleotides are further present at a surface density sufficient so that the conjugates are stable.
  - 438. The conjugates of claim 437 wherein the oligonucleotides are present on

WO 03/51/65

PCT/USBLEIT191

surface of the nanoparticles at a surface density of at least 10 picomoles/cm²

- 439. The occlugates of claim 438 wherein the eligorauleutides are present on surface of the autoparticles at a surface density of at least 15 piccasoles/em<sup>2</sup>.
  - 440. The conjugates of claim 459 wherein the oligonocleotides are present on surface of the manaparticles at a surface density of from about 15 picomoles/em<sup>2</sup> to about 40 picomoles/em<sup>2</sup>.
- 10 441. The conjugates of claims 435 or 436 wherein the nanoparticles are metal nanoparticles or remiscanductor nanoparticles.
- The conjugates of chien 441 wherein the nanoparticles are gold nanoparticles.
- 44). The conjugates of claims 435 or 436 wherein the objects/cited compiles at least one type of proceptition object-bodded, the recognition portion having a sequence complementary to at least one portion of the sequence of a models cold or modified objects/cited.
- 444. The coclugates of claim 443 wherein such of the recognition oligometerides comprising a specer portion and a recognition portion, the spacer portion being designed so that it is bound to the nanoparticles.
- 25 445. The conjugates of claim 444 wherein the spacer portion has a molety constantly bound to it, the molety comprising a cyclic disulfide functional group through which the spacer portion is bound to the necesparticles.

WO 03/51665

PC/DUSHARI 190

- 446. The conjugates of claim 464 wherein the spacer portion has a moiety covalently bound to it, the moiety comprising a polythrol functional group through which the spacer portion is bound to the nanoparticles.
- The conjugates of claim 442 wherein the spacer portion comprises at least about 10 gusteotides.
- 448. The conjugates of claim 447 wherein the spacer portion comprises from about 10 to about 30 auctootides.
- 449. The conjugates of claims 448 wherein the bases of the nucleosides of the spacer portion are all adentace, all threnines, all cytosines, all unarils or all gazanines.
- 450. The conjugates of claims 435 or 436 further a type of diluent 15 oligonucleotides.
- 451. The excepanticles of claim 450 wherein the diluent oligomethodides contain about the same number of nucleotides as are contained in the spacer positions of the recognition oligomotic tubes.
- AS2. The manoparticles of claim 451 wherein the sequence of the diluent oligonocleoties is the same as that of the spacer pertions of the recognition olimonucleutides.
- 453. A method of binding oligomelectides to unoparticles to produce manoparticle-oligomelectide conjugates, the method comprising:
  - proxiding oligonucloctides baving covalently bound cyclic disulfide function groups that can hind to manoparticles; and

PCTOUSHIJH 190

contacting the oligonacteorides and the nanoparticles under conditions offsetive to allow at least some of the oligonacteorides to bind to the maneparticles to produce the sunoparticle-oligonacteoride conjugates.

- 5 454. A method of binding oligonucleoides to nanoparticles to produce nanoparticle-oligonucleoide conjugates, the method comprising:
- providing oligonucleosides having covalently bound polydrial function groups that can bind to unropasticles; and
- contacting the oligonucleotides and the nanoparticles under conditions

  10 effective to allow at least some of the oligonucleotides to bind to the nanoparticles to produce the zamparticle-oligonucleotide conjugates.
  - 455. The method of citizes 454 or 455 wherein the nanoparticles are metal nanoparticles or somiconductor nanoparticles.
    - 456. The method of claim 455 wherein the nanoparticles are gold nanoparticles.
- 457. The method of claims 453 or 454 wherein, the oligomedicolidate comprising at least our type of acceptation oligomedicolidate, each of the recognition of agreement of the comprising a gooder portion and a recognition portion, the spacer portion having a molecy covatestly bound thereto, the molecy comprising a financiously group which can both to be exceptively.
- 452 The method of claims 457 wherein the spacer portion comprises at least 25 about 10 pucketides.
  - 459. The method of claims 458 wherein the spacer portion comprises from about 10 to about 30 nucleotides.

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- 460. The method of claims 459 wherein the bases of the nucleotides of the spacer are all adenines, all thymnices, all cytosines, all uracills, or all guagines.
- 5 461. The costed of claim 457, wherein the oligonoschoolists further comprising a type of dilutest oligonoschoolists and contacting the oligonoschoolists with the anappraisite under conditions effective to allow at later zonce of each of the types of oligonoschoolists to bind to the nanoparticles to produce the nanoparticle-eligonoschoolide conjugator.
  - 462. The method of claim 461 wherein the dilucer oligonucleotides contain about the same number of nucleotides as see contained in the spacer pertions of the recognition oligonucleotides.
- 463. The method of claim 462 wherein the sequence of the diluent oligenucleotides is the same as the sequence of the spacer portions of the recognition oligenucleotides.
- 464. The method of claim 457 wherein the oligonucleotides comprise at least 20 two types of recognition oligonucleotides.
  - 465. A method of binding oligonucleotides to charged nanoparticles to produce assoperticle-ollgosucleotide conjugates, the method comprising:
- providing oligonucleotides having covalently bound cyclic disulfide 25 fuzetion groups that can bind to managerticles, the oligonucleotides comprising:

a type of recognition oligonucleotides; and a type of diluent oligonucleotides;

contacting the oligonselectides with the nanoparticles in water for a period

PCT/USUL/UT 150

of time sufficient to allow at least some of each of the types of oligonacteristics to bind to

edding at least one salt to the water to form a salt polition, the ionic strength of the salt solution being sufficient to overcome at least partially the electrostatic saltestion or repulsion of the oligomeatesides for the sanoparticles and the electrostatic repulsion of the oligomeatesides for each other; and

contacting the oligoportleotides and paraphricies in the salt solution for an additional period of time sufficient to allow additional oligoportleotides of each of the types of oligomecleotides to bind to the nanopuralists to produce the nanoparticaoligomecleotide copinguises.

466 A method of binding oligonucleotides to charged paneparticles to produce nanoparticle-oligonucleotide conjugates, the method comprising:

providing objects baving covalently bound polythiol function 15 groups that on bind to nunopasticles, the objects to comprising:

a type of secognition oligometeorists; and

a type of diluent oligonucleolides;

contacting the oligenuclooides with the asseparateles in water for a period of time sufficient to allow at least some of each of the types of oligenucleoides to bind to 20 the asseparateles;

adding at least one salt to the warr to form a salt solution, the ionic strength of the salt solution bring sufficient to overcome at least partially the electrostatic attraction or regulation of the oligonouscedides for the nanoparticles and the electrosistic regulation of the oligonouscedides for each other, and

contacting the oligementeodides and supposations in the salt solution for an additional period of time sufficient to allow additional oligementeodides of each of the types of oligementeodides to bind to the nanoparticles to produce the nanoparticle-objementeodides conjugates.

WO 03/51/45

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- 467. The method of choice 465 or 466 wherein the nanoparticles are metal nanoparticles or servicenductor nanoparticles.
- 5 468. The method of claims 467 whorein the nanoparticles are gold nanoparticles.
- 469. The method of claims 465 or 466 wherein all of the salt is added to the water in a single addition.
- 410. The method of claims 465 or 466 wherein the salt is added gradually over
- 471. The method of claims 465 or 466 wherein the salt is selected from the 15 group constitting of solders etholeds, transparktum oblecteds, postations oblected, autocolium, childreds, polium, souther, autocolium actume, combination of two or more of these salts, one of those salts in a phosphore buffer, and a combination of two or more these tatis in a obsorbable buffer.
- 20 472. The method of classs 471 wherein the salt is sodium chloride in a phosphate buffer.
- 473 The method of claims 465 or 466 wherein nanoparticle-oligonucleotide conjugates are produced which have the oligonucleotides are protent on surface of the manoparticles at a nurface density of at least 10 picomolectral.
  - 474. The method of claim 473 wherein the oligonucleotides are present on surface of the nanoparticles at a surface density of at least 15 picomoles/om<sup>2</sup>.

WO 01/81/45

PCT/USHAII 150

- 475. The method of claims 474 wherein the objyountleotides are present on surface of the nanoparticles at a surface deasity of from about 15 picomoleulem<sup>2</sup> to about 40 picomoleulem<sup>3</sup>.
- 476. The method of claim 465 wherein catch of the rocognition of geometeorides comprises a spacer portion and a recognition portion, the aspoon portion having attached to it the moiety comprising a cyclic disalfale functional group which can bind to the amoparticles.
- 477. The method of cloim 466 wherein each of the recognition oligosuclostides comprises a spacer portion and a recognition portion, the spacer portion having structure to it the molecy comprising a polythiol functional group which can bind to the managentitides.
- 478. The method of claims 476 or 477 wherein the spacer portion comprises at least about 10 procloodides.
- 479. The method of obtim 478 wherein the spacer portion comprises from about 20 10 to about 30 studentides.
  - 480. The method of claims 476 or 477 wherein the bases of the nucleotides of the spacers are all oderines, all thymines, all cytosines, all tracils, or all guarantes.
  - 5 481. The method of claims 476 or 477 wherein the dilutal oligomeolootides contain about the serve number of nucleotides as are contained in the spacer portions of the recognition oligonucleotides.

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- 482. The method of classa 481 wherein the sequence of the dilucat oligonuclectides is the same as the sequence of the spacer portions of the recognition oligonucleotides.
- 483. The method of claims 476 or 477 wherein the oligonucleotides comprise at least two types of prognition oligonucleotides.
- 484. Objectives having a covalently bound cyclic disulfide functional group that can bind to the nanoparticles.
- 485. Oligonucleotides having a covalently bound polythini functional group that can bind to the manoparticles.
- 486. The compositions according to claims 433, 435, 445, 446, 453, 465, and 15 484 wherein a large hydrophobic group is located between the oligosusclookide and the cyclic distribution functional group.

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FIG.1

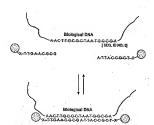
( seq. ip no: 3) X-C-C-T-G-A-G-A-T-T-T-C-C-C-T-G

G-A-A-C-T-C-T-A-A-G-G-G-A-G-X

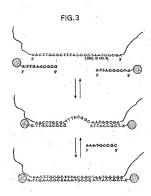
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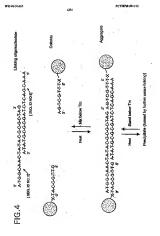
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FIG.2



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Modification with

FIG. 5

All haroparticles

Modification with

F high TACCOTTS 9

Addison of thicking RNA duplex

F AGTOCOTA'S Tailed

Addison of thicking RNA duplex

F AGTOCOTA'S Tailed

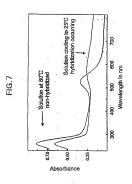
Addison of thicking RNA duplex

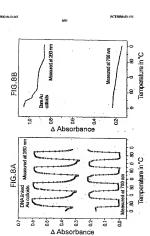
A Puriter or liquinger transform

and setting

FIG.6A FIG.6B FIG.6C

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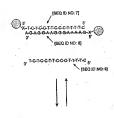
FIG.9A



FIG.9B

PCT/0501/01190

FIG.10





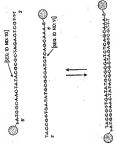


FIG.11

FIG. 12A
Combination limit

SEQ NONE;

PTCGTACCACCTATOC THTOCTGACATCGCC

FIG. 12B
PROBLEMBERS

PTCGTACCACCTATCC THTOCTGACATCGCC

FIG. 12B
PTCGTACCACCTATCC THTOCTGACATCGCC

FIG. 2C

Install size

FIG. 12C

Install size

FIG. 12C

Install size

FIG. 12C

Install size

FIG. 12C

FIG. 12C

Install size

FIG. 12C

F

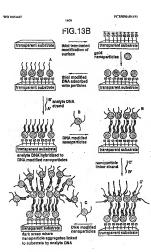
WO 01/51665

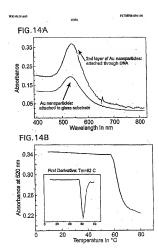
FIG.13A











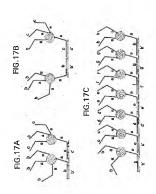
PCTASSIAH 199

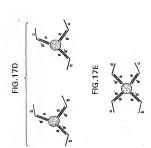


FIG.16A 24 Base Template
strictor and control of stric

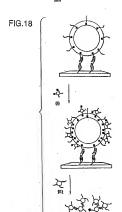
FIG. 1 6B 4g Base Templete with Complementary 24 Base Ellier sometimes of the controlled of the contro

FIG. 16C 72 Base Template with Complementary 48 Base Tiller systems are systems are some process and systems are some process and systems are some systems.





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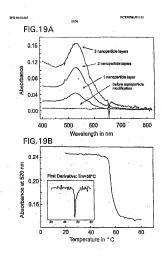


FIG. 20A

FIG. 20B

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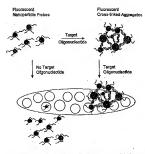




FIGURE

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FIGURE SA



The fluorescent nanoparticle probes pass through the membrane The fluorescent cross-linked aggregates are retained by the membrane

WQ 01/51665

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## Anthrax PCR Product

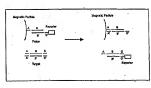
STE COC GAT GAG TICA GTA GTT AAG GAG GCT CAT AGA GAA GTA ATT AAT STE COC CTA CTC AGT CAT CAA TYC CTC COA GTA TCT CTT CAT TAA TTA TOG TOA ACA GAG GGA THA THO THA ANT ATT BAY AAG GAT ATA AGA AAA AGG AGT TOT CTC CCT ANT AAG AAT THA TAA GTA THC CTA TAT TCT TIT ATA THA TOO AGE GIT ATA THE TAG ANA THE AND ATA CTE AND ORD IT IS TAT AND AGE TOO CAN TAY AND ATO TITY AND THE TAY CAN THE COS AN IS

141 mer Anthrew PCR product [SS Q To 130:56]

3 TTA TAA CTA TEC CTA CO. 3 T C CSE 9 16 No. 3 T C [589 10 HO: 51]

FIGURE 23

WO NICHAEL BY SAME Probe PCCRESSION SO NICHAEL SO NICHA



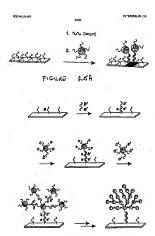
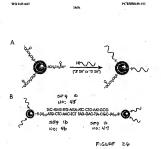
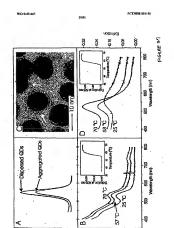


FIGURE 850





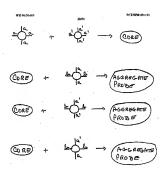
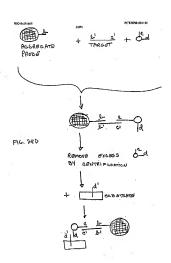


FIGURE 28A

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. FIGURE AT B



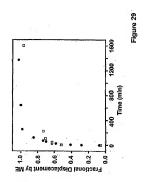
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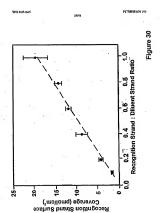
Three or + to

FIGURE 28E

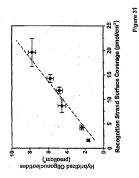


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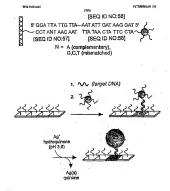


Figure 32

WQ 01/51665

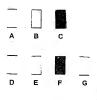
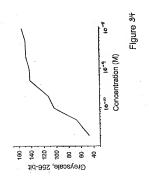


Figure 33

WO 91/51665 PCT/RES01/



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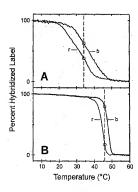


Figure 35

CATG

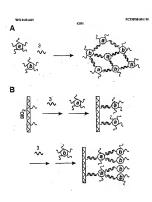


Figure 37

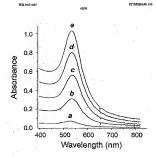


Figure 38A

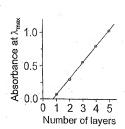


Figure 38B

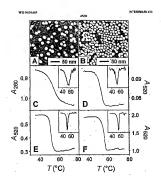
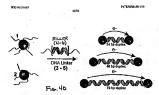
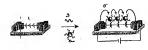
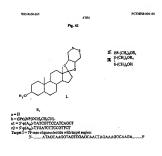


Figure 39



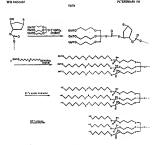




DCTERSOLD1100

PCT/ISDI/01150

 $R_{\rm p}$  = hydrogen, as sikyl group, an eryl group, or a substituted sikyl or aryl group  $R_q = an$  attached oligonucleotide or modified oligonucleotide



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# SECONDE PIENING

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# 【国際公開パンフレット (コレクトバージョン)】

(12) INTERNATIONAL APPLICATION PT BASHEST PRICE THE PATENT CHOPERATION CREATY (PCT)

## CORRECTED VERSION

(7) World Confinence Progeny Organization
Monotonery Organization
(A) Neurostander State
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9879-000-000

19th Application NANOSHTERISE INC. IT SUSS 2881 Mayor Anamas Facinism 8, 1006.

19th Language NANOSHTERISE INC. IT SUSS 2881 Mayor Anamas Anamas 8, 1006.

19th Language NANOSHTERISE INC. IT SUSS 2881 Mayor Anamas Anamas

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\$ (54) Title: NANOPARTICIAS HAVING OF IGONECLEGIBLES AFFACHED THERETO AND REFS THEREFOR

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WO 01/03/645

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PCT/US04/04150

### NANOPARTICLES HAVING OLIGONUCLEOTIDES ATTACHED THERETO AND USES THEREFOR

This investion was made with government support under National Institutes
Of Health (NIII) grant GM10265 and Army Research Office (ARO) grant DAAG550667-1-0133. The programment has certain rights in the invention.

10 This application is a consumerate-le-part of paneling application number (02/14/4/67, Incl.) mo. 25, 1999, which was a confinantion in join of possibility application number 002/40/35, filed January 29, 1999, which was a continuation-inpart of pending PCT application PCTLX599/12/128, which was filed high 21, 1997, which are incompared by reference. Benefit of provisionin application non-15 600311499, Filed July 29, 1999, 602001491, filed July 102, 2000; 6021145409, Benefit James V J. 35,000 his or dained, the deliverate are incompossible by ordenium.

## FIELD OF THE INVENTION

The invention relates to useshods of descring nucleic usids, whether natural or ayuthecis, and whether modified or ususoidified. The inventions has relates to materials for descring nucleic acids and methods of making house interiors. The invention further relates to methods of man-fahrication. Fuelly, the invention relates to method of repressing a selection business soft from one moderate to method of repressing a selection business soft from other moderate.

### BACKGROUND OF THE INVENTION

The development of matters for descriting and sequential problem acids in cinical to the diagnosis of genetic, besterials, not viral diseases. See Mansfelda II. Set al. Motionals and Calabate Problem, § 164-156 (1997). A proserve, there are a variety of matter of the control of the control

### WO 01/05/565

### PCT/USOM/01190

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## SUMMARY OF THE INVENTION

The invention provides mediads of detecting nucleic colds. In one

one embediment, for multiple consprises consisting a reachir acid with a type of

napoparticles having oligonucleotides attached thereto (manoparticle-oligonucleotide)

:

PCT/LS0L01190

conjugates). The nutricis acid his at least two particus, and the oligometeodrises on each magnetisk line a sequence complementary to the sequences of at least two portions of the nutricise said. The conducting takes place under confidence effective to allow hybridization of the off-generateodrise on the nanoparticles with the nucleic carid. 5. The hybridization of the off-generateodrise on the nanoparticles with the nucleic acid results in a destrainth change.

In author coincidence, the method comprises contenting a medics said with a least to the past disrupcificide having disprachedited strated dresses. The allowance of the first type of amorphism having of a subject to the first type of amorphism having on the first type of amorphism content type of amorphism have been supposed to the past of the disprachedited on the second-type of amorphism have been supposed to the content of the disprachedited on the second-type of amorphism have been supposed to the content of the content o

3. In a feether embodiesses, the models compine providing a walknown having a feet type of management is standed theme. The first type of management is standed theme. The first type of management is standed themes, not the eligenationists have a requested and the experimental control of providing of the prov

point. The nesthod may facther comperies providing a binding eligenselectiols having a selection sequence having at least two portions. He days period to bring complementary is a least a specin on of the conjunction of the propriete solution on the second type of manuparticles. The binding oil gorounicoside is contacted with the accord type of manuparticle-diligonacteristic conjugates bound to the substrate under IN TRANSMIRM ISO

conditions effective to allow hybridization of the binding oligonucleoside to the oligonucleosides on the nanoparakide. Then, a limit type of interpretation sharing oligonucleosides maked therein, the oligonucleosides having an expense complementary to the sequence of a second portion of the binding oligonucleoside, is 5 contented with the binding oligonucleoside became all use substitute under conditions artificial to allow the binding oligonucleoside became allow understood to the disconnection of the conditions artificial to allow their states of the disconnection of the disconnect

contracts with the binding offigment to like between 10 the substitute under conditions effective to a live hybridization of the binding offigment cold to the objectivelessistics on the nanoparticles. Finally, the descrable change produces by these hybridizations is observed.

In yet another embodiment, the method comprises contracting a markets acid

10 with, submark breing oligomechades whated tronce, no oligomechades without present and present an expension of the present yes a few proposed of the sequence of the nuclear skell select under confidence effective to other hydridisation of the object-confidence of the fast type of consputation, the constrainty objects object-confidence of the object-confidence of the fast type of consputation, the constrainty objects object of the object-confidence of the fast type of consputation, the constrainty objects object-confidence of the object-c

on the first and accord types of neospaticite. Finally, a descendoic change producted 25 by these hybridirations is observed. In socious resubdirectat, the nesthod comprison contrasting a macinic said with a substrain having ofiground-coides attended financia, for oligonacidorida kaving a secure occupiencetary to a first portion of the sequence of the modific acid. The contesting lates above under considerations effective to allow by indicatation of the productions of the contrasting lates.

30 ofigonocleolides on the substrate with the nucleic acid. Then, the nucleic sold bound to the substrate is contacted with hipocontes having ofigouselectides attached thereto,

#### WO 02/051665

#### PCT/ILN01/01 (50)

the alignnucionistics having a sequence complementary to a portion of the sequence of the nuclei and. This contacting takes place under conditions effective to allow hybridization of the olignnucleotistic on the liposomes with the nucleic acid. Next, the liposome of ligourcelotistic conjugates beingt in the substants are contacted with a

- 5 first type of incorporations having at least a first type of oligorunderoides attached thereto. The first type of oligorunderoides have a hydrophobic group statched to the one of outstached to the anneymentable, and the constanting sizes place tunder conditions effective to allow autochannet of the oligorunderoides on the opportunities to the lipocomes as a result of hydrophobic internetions. A detectable change may be
- 10 observable at this point. The method may further comprise overselling the first type of amougnated-object-coolede copingstes bound to the liposence with a second type of amougnation being objective studied between C. The first type of temporarilles have a second type of objective cooledes attached thereto which have a sequence complementary on a text as protoco for the sequence of the objective of the objective confidence on the complementary on a text as protoco of the sequence of the objective confidence on the
- 15 second type of tanoparticles, and the oligomeleouides on the second type of anapparticles having a sequence complementary to a least a portion of the sequence of the second so the first type of neutroparticles. The contacting takes place under conditions efficient to allow hybridisation of the objective on the first read according to the contacting takes place under conditions efficient to allow hybridisation of the objective on the first read according to the contact to the
- In untilize unbindliment, the midmal comprises contenting a market acid to be detected with a statute being displaced as statuted to the detected with a statute being displaced as forced in the property of the origination of the management of the
- contacting takes place under conditions effective to ollow hybridication of the 30 oligonsuleolides on the numoparticles web said nucleic acid. Then, the substrate is

### WO 01/05/1665

PCT/US01/01190

confected with silver stale to produce a descutable change, and the detectable change is observed.

- In yet another embodiment, the method comprises providing a substrate teaching a first type of nanoparticles attached thereto. The manoparticles have 5 oligonucleotides attached thereto, the oligonucleotides having a sequence
- organizations statement unersety, are Organizationstates are up a September complementary to effect performed has bequence of a matter local to the substant Then, the nucleic solid in contrasted with the nunoparticles attached to the substant under conditions effective to allow hybridization of the oligonoscicotics on the anteroparticles with and nucleic acids. Note, an aggregate probe comprising at least
- 10 was types of monopraticals having oligonucleosides standard distracts is provided. The manoparticiss of the aggregate peobe are bound to each other as a result of the hybridization of some of the oligonucleosides anatheris to litera. At least one of the types of manoparticles of the aggregate peobe have oligonucleosides standard which layer as course complementary to a second perior of the sequence of each of the course of the course of the course of the course of the sequence of the
- 15 muchic acid. Finally, and nucleic acid bound to the substrate is contracted with the aggregate probe seader conditions effective to allow hybridization of the oligonoclouddes on the aggregate probe with soid nucleic acid, and a detectable change is observed.
- to inform conductions, the method comprises providing a substance having position of the configuration of the compression of the compression of the complementary to a first period or first superiod or if method to be different. An aggregate probe comprising at least two types of immorphish abusing objective structure describe in provided. The memorphish abusing objects attributed structure describe in provided. The memorphish of the aggregate probe comprising to one other than a reason of the type of the compression of the aggregate probe are because to one other than a reason of the polyholication of common of the
- 25 oligorosiosolides statubolos o disura. At lasar ono of the types of casospositishes of the agetypase pocie here oligorositeotides attached thereto which have a soponese complementary to a second portion of the sequence of sind metales such. The metales sold, the subsents and the aggregate peobe are contexted under conditions effective to allow hybridisticulor of suit antestica and with the oligoroccides in on the aggregate.
- 30 probe and with the oligonucleotides on the substrate, and a detectable change to observed.

#### WO BURGES

### PM-T/II SA1401190

In a farther trobodamout, the method comprises providing a substant having oligents/horides statched threato. An aggregate probe comprising at least two types of europarticles having oil geometeotides attached therete is provided. The sunoparticles of the aggregate probe are bound to seek other as a result of the

- 5 hybridization of some of the obligonoclosides untarbel to from. A league one of the types of amopuration of the aggregate puch is an obligonoclosides wranched thereto which have a sequence complementary in a first partie of the nequence of a musclet said to be detected. A type of managarides having at least two types of disponactional stathed there is provided Th first type of oligonoclosides has a
- 10 sequence complementary to a second portion of the sequence of said smetric acid, and the second type of disjunctionists has a sequence complementary to at least a portion of the acqueres of the disjunctionistic factor and the substrates. The notionist, the significant control of the substrates of the substrates of the substrate and the said of the substrates of the notion of the substrate are consistent under control of the substrate are consistent under the substrate and substrate
- 15 oligonuclootides on the aggregate probe and on the transparticles and hybridization of the oligonuclootides on the nanoparticles with the oligonizationides on the substrate, and a detoritable change is observed.
- In another embodiment, the method comprises contacting a muchic acid to be detected with a substrate having ollopscate-louder strucked detected. The oligonacieoides have a sequence complementary to a first portion of the sequence of add nucleic coul. The contracting takes place used re conflictors effective to allow
- hybridization of the oligonucleonices as the substrate with said murities and. The markets acid hound to the substrate is contacted with lipocomes having oligonucleotides ettached thereto, the oligonucleotides having a sequence
- 25 complementary is a perform of the requestor of add succle acid. The constraints the place under conditions effective to allow byhrdination of the oligonosciousless on the liposomers with raid nucleic succle Ann aggregate pube comprising at frest two types of amorpatricles having oligonoclosides alteriod thresho is provided. The assessment of the aggregate pube are bound to each dure as a result of the
- 30 hybridization of some of the oligonucleotides attached to them, at least one of the types of nanuparticles of the aggregate probe having oligonucleotides attached thereto

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which have a hydrophobic group anathes to the end not attached to the manoparticles. The figureous bound to the substante are contracted with the aggregate probe underconditions effective to allow attachment of the oligometodise on the aggregate probe to the figureous a result of hydrophobic interactions, and a descendible

5 change is observed. In yet another embediment, the method comprises providing a substrate having oligonacleotides attached thereto. The oligonocleotides having a sequence complementary to a first portion of the sequence of a nucleic acid to be detected. A core probe comprising at least two types of nanoparticles is provided. Each type of 10 msnoperticles has ofiguracleotides attached thereto which are complementary to the oligonacionides on at least one of the other types of nanoparticles. The nanoparticles of the aggregate probe are bound to each other as a result of the hybridization of the oligonusicalides attached to them. Next, a type of nanoparticles having two types of oligonucleotides attached thereto is provided. The first type of oligonucleotides has a 15 sequence complementary to a second portion of the sequence of said nucleic acid, and the second type of oligonucleotides has a sequence complementary to a portion of the sequence of the alignmecleutides attached to at least one of the types of nanoparticles of the cure probe. The nucleic enid, the nanoparticles, the substrate and the core probe are contacted under conditions effective to allow hybridization of said machine 20 acid with the olimosus leotides on the nanoparticles and with the oligorascleotides on the substrate and to allow hybridization of the oligonactorides on the nanoparticles with the oligenucleotides on the core probe, and a detectable change is observed. Another embediment of the method comprises providing a substrate having

oligomotocillas derholds dience, the eligomotocillas having a response complementary to a find purision of the aspect coeff a motifie acid to be descente. A one probe compression at text error types of manoparticles is provided. Each type of manoparticles has oligomotocilcolois attacted fueners which me complementary to the collegomotocilcolor or in these cost own types of manoparticles. Of the compression of the oligomotocilcolor or in these cost own types of manoparticles. The compression of the superior probe are bound to each other on a result of the hybrid inviting of oligomotocilcolor compression.

sequence complementary to a second portion of the sequence of said nucleic acid and

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a sequence complementary to a portion of the nequence of the oligonorcheotides structured to ut least one of the types of manageriticles of the core probe as provided. The nucleic acid, the linking oligonorcheotides, the substants and the core probe are consusted under conditions effective to allow hybriduation of said markin calci with

- 5 the linking oligenucleotides and with the oligonucleotides on the substrate and to allow hybridization of the oligonucleotides on the linking oligonucleotides with the oligonucleotides on the core probe, and a detectable change is observed.
- In yea united confodience, the method comprises proving an expertised in whice of programs of each study of them on all configure on corn only see of Meding 10 originate configure or consistent of the second configuration of the province of case purious in complementary to the sequence of one or the origination of the expenses of the origination of the or
- oignustactions are contented mades conditions at Retains to allow hydridization of the binding objects contented in the materials noted. There, a detectable change is observed. The management-objects decopying areas may be contacted with the 20 binding oligonuscheolides price to being contacted with the matches noted, or all there may be contented simultaneously.
  - In another embediment, the method comprises controling a succleic acid with at least two types of particles having oligonuclostidas attached thereto. The oligonuclostidas on the first type of particles have a sequence complementary to a
- 25 little portion of the sequence of the muchic said and have energy door molecules on the walds not attached to the particles. The ollipsoucciosides on the occased type of particles have a sequence complementary or a zerood partition of the sequence of the markets said and have energy secreptor molecular on the rank not statished to the particles. The constrainty lakes place ower soundiness referred to at law hybridistantion.
- 30 of the oligonucleotides on the particles with the nucleic acid, and a detectable change

#### INTERNATION (SO

brought about by this hybridization is observed. The energy donor and acceptor molecules may be illustrated molecules.

In a feature controllement, the method comprises providing a type of interest productions between the controllement and the controll

of the uncleic acid. The nucleic acid is contained with the microsystems and the nanopasticles under conditions effective to allow hybridization of the oligonucleosides on the later microsystems and on the nanoparticles with the nucleic acid. Then, changes in fluorescence, another detectable olongs, or both are observed.

In another embodiment, the mothod comprises providing a first type of metallic or acmiconductor nanoparticles having ofigomotocides stracked thereto

netation or amountainer averagemente inviting objurnation/less association and The objurnation-less was requiremented to a finite or an extension of the nequence of the models and are hished with a fibureact to ratio called a type of sentill or a recolorabation assequated harding objurnation and thereon is also provided. These objurnationals having objurnational assemble thereon is also provided. These objurnation and in the sent as expected completeneously to a second protein of the sequence of the medical and of our two two facilities with a 20 flowtracter models. The models deal for sentiated with the very constraint which the very provided to 20 flowtracter models. The models deal for sentiated with the very constraint which the very provided to 20 flowtracter models. The models deal for sentiated with the very constraint which the very provided to 20 flowtracter models. The models deal for sentiated with the very constraint which the very provided to 20 flowtracter models. The models deal for sentiated with the very provided to 20 flowtracter models. The models and the models are constraint when the very provided to 20 flowtracter models. The models deal for sentiated with the very provided to 20 flowtracter models. The models and the very provided the very provided the very provided to 20 flowtracter models. The models are the sentiated with the very provided the sentiated of the very provided to the very provided the very pr

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15 In a further embodiment, the sentent comprises providing a type of particle bearing alignmentation shaulded theses. The dispursable of the sent and sentent provides a shauld these the a find particle of the sentent particle of the sentent end as accord particle, to his provides a find a resource of the motion and A. Spee of providing sentent provides and at assenting opticals in also provides. The first protect in and a resource complementary to the first position from the sentent provides and at the first position of the collimentations sentent to the previous of soften protection and the first position of the sequences of the motion set of the sentent sent of the protection and the sentent provides and the sentent protection and the sentent protection and the sentent protection are sentent provides and the sentent protection are sentent protections are sentent protections.

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particles and the probe oligonocleotides we contacted under conditions effective to allow for hybridazation of the oligonocleotides as the particles with the probe oligonocleotides in produce, satisfies probe. Then, the sactility probe is contested with the machine acid under conditions efficient to provide for hybridization of the 5 markets under with the probe oligonocleotides. The particles are recoved and the

reporter motocute detected. In yet snother embodiment of the method of the invention, a nucleic acid is detected by contacting the nucleic ucid with a substrate having oligonucleotides attached thereto. The oligonucleorides have a sequence complementary to a first 10 portion of the sequence of the meleic acid. The oligonucleotides are located between a pair of electrodes located on the substrate. The contacting takes place under conditions effective to allow hybridization of the oligonactootides on the substrate with the mucloic sold. Then, the nucleic acid bound to the substrate, is connected with a type of nanoparticles. The nanoparticles are made of a material which can conduct 15 electricity. The nanoperticles will have one or more types of alignmelocaldes attached to them, at least one of the types of niigonucleotides having a sequence complementary to a second portion of the sequence of the nucleic acid. The contacting takes place under confinious effective to allow hybridization of the obsermeteetides on the nanoparticles with the nucleic acid. If the nucleic seid is 20 present, a change in conductivity can be detected. In a preferred embodiment, the substrate will have a plurality of pairs of electrodes located on it in an erray to allow for the detection of multiple partions of a single mucleic acid, the detection of smiltiple different nucleic oxids, or both. Each of the pairs of electrodes in the array will have a type of oligonacleotides attached to the substrate between the two electrodes.

25 The invention further provides a method of detecting a nucleic acid whereint he method is performed on a substrate. The method comprises detecting the presence, quantity or both, of the nucleic acid with an optical seamor.

The investion further provides kits for descring suc-leic soids. In one cabbodiment, the kit comprises at least one container, the centiliary holding at least 30 two types of unonperticles faving oligonesclosides attached thereto. The oligonesclosides on the first type of own-particles have a cognume complementary to

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the sequence of a first portice of a nucleo acid. The oligonucleotides on the secural type of minoparticles have a sequence complementary to the sequence of a second portion of the nucleic acid.

Alteratively, the kit may compele at least two containers. The first containers

5 holds monoparishors having subject-toolsides stranded factors which have a sequence
complementary to the expenser of all gardinori for a sundice, such less record
containor holds nanoquarishes them sing aliquosateoisticit such such all her second
containor holds nanoquarishes them sing aliquosateoisticit such such all her some which have a
sequence complementary in the sequence of a second portion of the mellors of
the single second containers. The

In a further embodiment, the list comprises at least one container. The combinior holder is realized or estimization consoperation having ellipsymatesistics attached thereto. The oligomethesistics have a sequence comprises may be pretion of a material and have flaurescent molecules attached to the ends of the oligonationation on an attached to the mospatificiar.

1,5 when confidence on bodience, the left comprises as solutions, the admirest having tacked flow one competition, the imagenites below on Ejecution of the Confidence of the

In another embodiness, the list competes a substrate having diagnost-conideratuched thereto which have a sequence complementary to the sequence of a first perion of a matetie seed, a flat eccutaions bodding amorpurches having generated the seed of the second perion of the sequence of a first period sequence of a second perion of the material residue and second commitme the billing sequence of a second period on the material residue and second commitme thebling PCT/US01/01190

complementary to at beast a portion of the oligenselectides attached to the nanoparticles in the first container.

- In you enotine repolationes, the left comprises a substitute, a filts container hadding amounteristic a encond contention reliefung in structure of impostmentations of the structure of the contention and the contention and
- 10 In a further condecisional, the RC comprises a relational hereing objective values that the latest with these as expense comprisements by the expectate of a first position of a mariest soil. The list data instants a forst execution to support on the position of a mariest soil. The list data instants a forst execution to support on the section of the sequence of a second perion of the section will and a second consumer bridging 15 manageainties having as least a fast type of "disposacionistics brings in the latest and the section of the second transport of the section in the section of the sect
- second type of managamicish having alignmaticesities strated threse, his 20 oilgeomideoides having a sequence complexentisty to at least a partism of the sequence of a second type of olignmateriorists strateds to the first type of samoparticist. This second type of olignmateriorists strateds to the first type of samoparticist have a sequence samplementary to the sequence of the oligomethorists on the second type of amagnetists.
- 25 In aussither embodiment, the lax comprises a who-tast having amorparticles statisched to it. The nanoparticles have objectiveles of the configuration of the section of the model of the model, the lax sequences complementary to the respective of a first provise of a model in acide. The lat also includes a litest continue hability are aggregate probe. The aggregated epole comprises at least two types of semegated having long-inconclusions amonthed to
- 30 them. The correspondictes of the aggregate probe are bound to each other as a wealt of the hybridization of some of the oligenucleotides attached to each of them. At least

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one of the types of nanoparticles of the aggregate probe has oligometicodides attached to it which have a sequence complementary to a second portion of the sequence of the nucleic acid.

In yet another embodiment, the kit comprises a substrate having

- 5 oligonacierodese intende to Ni The oligonacieroles have a sequence complementary to the sequence of a first portion of a muchic acid. The kit further includes a first constitute holding in aggregate peole. The aggregate proble comprises at faunt two types of muoparticles having oligonacieroless attached to them. The amountariles of the aggregate poles at bound to each other as a retail of the hybriditation of some of
- 10 the oligonucleosides attached to each of them. At least one of the types of unoparticles of the aggregate probe has oligonucleosides attached thereto which have a sequence complementary to a second portion of the sequence of the nucleic acid. In an additional embodiment, the kie comprises a substrate having.
- oligementerondes attended to it was a final optimizer backling an aggregate pedec. The 15 aggregate probe comprises as least two types of companients having alignmented size assessable to them. The nanoparticles of the aggregate probe a robust to each other as a result of the hybridization of some of the olignmenteroldes attached to each of them. At least one of the types of managemental or the aggregate probe has olignmenteroldes matached to it which have a sequence complementary to a first protect of the expense.
- 30 of the macleic acid. The feet also includes a economic container holding remoperations. The manoperations have at least two types of oligonomic besides at exactles to them. The feet all type of officement consopheratories a second postume of the sequence of the mucleic mink. The second type of oligonomic boolides has a requeror complementary to all feet a position of the sequence of the oligonomic boolides analysis.
  - In another embodiment, the left comprises a substrate which has oligomedicatides attached to it. The oligomedicatides have a sequence complementary to the sequence of a first portion of a nucleio said. The kill also comprises a first container holding liposemes between glossessebonifies attached to them. The
- 30 ofigonucleorides have a sequence complementary to the sequence of a second portion of the nucleur axid. The kit further includes a second container holding an aggregate

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probe comprising at least two types of nanoparticles having oligomechaedee strucked to dism. The atmosphisics of the aggregate probe are bound to each other as a result of the hybridistics of some of the designation of some of the designation of some of the legislation of the special control of the Articles one of the types of nanoparticles of the aggregate probe has oligomachaddes strucked 5 to it which hive a hydroghobic groups standed to the mole out standed to the managaments.

In a further embodiment, the kit may comprise a first container holding nanoparticles having oligonucleotides attached thereto. The kir also includes one or more additional containers, each container holding a binding oligonacleotide. Each 10 binding oligomotivoide has a first partion which has a sequence complementary to at least a portion of the sequence of alignmolecoides on the nanoparticles and a second portion which has a sequence complementary to the sequence of a portion of a nucleic said to be detected. The sequences of the second partions of the binding oligonucleotides may be different as long as each sequence is complementary to a 15 pertion of the sequence of the nucleic soid to be detected. In another embodiment, the kit comprises a container holding one type of manoparticles having oligonsuleotides attached thereto and one or more types of binding oligonucleotides. Back of the types of hinding oligonechoolides has a sequence comprising at least two portions. The first portion is complementary to the erquence of the oligonacleotides 20 on the nanoparticles, whereby the binding oligoracteotides are hybridized to the oligenucleotides on the nanoparticles in the container(s). The second portion is complementary to the sequence of a portion of the mucleic acid.

In sentire embedienes, bit may compile on or two continent balling we types of particles. The first type of particles having objectations desired policies and the particles and the particles are supposed or supplementary to the sequence of a first portion of a modern and. The objectations are their balled with an energy observe on the cash sent standard to the particles. The security oper particles being aliquent content attended to the particles. The security composition of a model of the particle seal. The objectation of a model of the particle seal. The security of the particle seal of t

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In a faither untheidismed, the lite comprises a first constaint building insequentials having eigenstatesides annheid textor. The lite late confusion or more additional continuous, well nonestime building infesting eitigensatestation. But in literature of the protein which has a sequence comprise confusion to the management of a protein which has a sequence of eigensatestation as the anomalism and second position which has a sequence or eigensatestary to the exquence of a protein of a second position which has a sequence complementary to the textor of the contract of the sequence of the second position of the interpolation of the sequence of the second position of the sequence of the material ends to destrocted. In yet under having one of the second position of the sequence of the material ends to destrocted. In yet under having disposational second position of the sequence of the material ends of the second position of the sequence of the material ends of the second position of the sequence of the second position of th

adigeneuclosidian uttached thereto and one or more types of binding oligonatelessists.

Each of the types of binding oligonatelosides has a sequence comprising at least two
portions. The first portion is complementary to the sequence comprising as least two
portions. The first portionia is complementary to the sequence of the oligonatelosides
on the nanoparticles, whereby the binding oligonatelosides are hydridized to the
oligonatelosides on the nanoparticles in the constancies.) The second portion is
complementary to the sequence of section of the nucleis and

In author internalise internalise internalises throughouse, the 1st computers at least three containers. The first considerable internalises internalises in the containers are considerable to first of edigmentation in territory as sequence complementary to the sequence of a first portion of a motion exist. The nit containers to the containers are complementary to the sequence of a second portion of the models seals. The hit may durather complies and micro considerables the bridge a sequence showing a varieties of expenses of the containers and the containers are not to the containers and the containers are not to the containers are not to the containers are not as a sequence of a second point or the complementary to the anaptive of the containers hading an objective clock between the complementary to the anaptives of the containers are not complementary to the anaptives of the containers are not complementary to the anaptives of the containers are not complementary to the anaptives of the containers and proposed cold containers.

In another cultiditions, the fit consprise one or two containers, the container(s) hadding two types of particles. The first type of puricitle having alignostications attended thereof that have a nequence complementary to a first. 30 persion of the sequence of a vanishe and and have energy donor embodes attended to the ends to the challed to the managements. It has exceed type of prairiefs whereigh the earth on the challed to the managements. It has exceed type of prairiefs whereight the containers are the challed to the containers.

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#### INTERTOTION

oligocuroficotides attached ricersto that have a sequence complementary to a second portion of the sequence of a medicia acid and have energy acceptor molecular attached to the cruds not attached to the sunspecificies. The energy denotes and soreptors may be fluoresteem inclusions.

5 In a furface embodiment, the bit comprises a first container healthing a type of microsystems having oligomechee/dock mateched flowers. The oligomechee/dock have a sequence complementary to a first patient of the requestor of a satiefie and and are lateled with in the rescuence molecular. The bit also comprises a second constant-bodding as type of acceptantible having depresentable in the bit of them. The looking as type of the respect took having depresentable in the production of the second portions of the sequence of the second portion of the sequence of the second portions.

In mother colordinate, the bit comprises a first consider a beliefuge flist type of motifies to remindentes management be having oligoment-order continues to the oligoment of the colorest continues to the colorest color

biolode with a flowcoment melandar.

De la maction embodieme, the let comprises a consistent healting are aggregate probe. The aggregate probe comprises as in sea two lyps of or insepartical tenders policyanucladed as made to have. The management of the aggregate benefit and the probest of the probest and the probest bound to each other as a result of the publications of time and the alignmentables assistant of the case of health publication of animal or the alignmentables assistant of the case of health publication of animal or the alignmentables standards the case of health publication of animal or the alignmentables probest of the adjustmentables as standards to a which sinve a sequence complementary in a protection of the augmentables as standards to a which sinve a sequence complementary in a protection of the augmentables as standards as a standard or a standard and a protection of the augmentables as standards as a protection of the augmentables as standards as a protection of the augmentables as a protection o

In an additional ambodiment, the kit comprises a container beliefing an against probe. The aggregate probe comprises at item two types of nanoparticles having oligonactionides attacked to them. The assoparticles of the aggregate probe of a membound to each other as result of the hybridization of some of the oligonacticosides attacked to seek of them. At item one of the types of assoparticles attacked to seek of them. At item one of the types of assoparticles

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of the aggregate probe has oligomeolootides ettached to it which have a hydrophobic group attached to the east not attached to the stanoparticles.

- In a further embodiment, the kit comprises a container holding a satellide probe. The satellite probe comprises a particle having attached thereto
- 5 oligorusclantides. The oligonoschooldes have a first portion and a second points, both, portions having requences complementary to portions of the outcames of an assistant. The setting through a loss compression proceed collegenoschooldes attended to the nanoparticles. The probe oligonoschooldes attended to the nanoparticles. The probe oligonoschooldes have a fine protein and a second portion. The first protein has a second protein active of the protein and a second portion. The distriptories of the segment of the protein can be a second portion and the protein of the protein and the p
- 0 the sequence of the flest portion of the oligonocheotide, whiched to the packetes, and both portions have sequences complementary to pertions of the sequence of the trucket and. The probe oligonocheotides also have a reporter molecule attached to one and.
- In another embodiment, the kit compelsing a continuer holding a comprobe,
  the occeptobe comprising at least two types of amospaticles turing oligonatelookides
  attached thereor, the nanoparticles of the core probe being bound as each other as a
  result of the hybridization of some of the oligonuteriolides obtached to them.
- In yet sandez embodiment, the kit comprises a substanta having statched to it at least one pair of destrokes, wide oligomeateroides attached to the substant between the electrodes. The oligomeateroides have a sequence complementary to a first portion of the exposmo of a multicle sold to be detected.
  - The invention also provides the satellite probe, an aggregate probe and a core
- This invention further provides a substante having nanoparticles statched

  25 theorete. The sanoparticles may have ollgomorileotites uttached therete which have a
  sequence complementary to the sequence of a first portion of a subcleie acid.
  - The invention also provides a metallic or sent/conductor nanopamicle having oligonucleotides enacted thereto. The oligonucleotides are labeled with fluorescent materials at the cuts not attached to the managemicle.
  - The invention further provides a method of nanofabrication. The method comprises providing at least one type of linking oligonarizedade having a selected.

### WO 01/05/1645

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sequence, the requeres of each type of finaling oligomorlecticle having at least two postions. The motion further comprises providing none or more types of anapstraticle having a ligomorlection attached thereto, the oligomorlecticles on each types of managements having a sequence complementary to a portion of the sequence of a 5 linking oligomorlectories. The inline oligomorlectories and anapstratics are constantly designed in the contraction of the sequence of a 5 linking oligomorlectories. The inline oligomorlectories and anapstratics are constantly designed output of the sequence of the sequence

thriting disposatestoste. The matting originate records and amount amount access was under considious effective to allow particiation of the disponuciesticae on the nanoparticles to the littling oligonucleotides so that a desired nanomaterials or consistentials in formed.

The invention provides another method of nanotherization. This method

10 ower providing. It least two types of an expectation to whose of presentation for a transfer of the management of the configuration of the configuration

The investion ferther provides runnematerials or manostructures composed of 20 nanoparticles bring oligo nucleotides attached thereto, the nanoparticles being hatd together by oligonucleotide connectors.

The two various also provides a composition comprising at least two types of temporalistic having object-looked state-find thereof. The object-lookeds con the first type of narrogatistic have a sequence complementary the respects of 2 first portion of a models exid or a linking object-looked. The object-lookeds on the second type of nanoparticles have a sequence complementary to the expected of a second particle of the societies deep designing object-lookeds.

The invention further provides an assembly of containers comprising a first container bottling renormities having oligomoticodes attached thereto, and a second container holding manuparticles having oligomatelecides attached thereto. The elisamoutoolides attached to the manuparticles in the first consistent here a sequence

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complementary to that of the oligonosclossides statehod to the nanoparticles in the second costsioner. The oligonusclossides affurbed to the nanoparticles in the second container have a requerior sumplementary to this of the oligonasclostides attached to the nanoparticles in the first container.

5 The invention also provides a nanoparticle having a plurality of different oligonucleotides attached to in.

The invention further questions a match of long-rating a solution disuscised to all water gast factors to protection from other returns its clied. The matthest comparison providing a control returns of managements and surprise of managements and the providing and control from the collegenciabilities on each of the types of managements having an acquerate complementary to the subsence of cond- for persons of this science materials and the control from the control for the control materials and the management of the collegenciations of the disquares cannot be made another confidence to all the "specification of the disquarescence and the managements with the admission and the control for the control control for the control of the control for the control of the control for the control of the co

In addition, the investion provides methods of making unique asseparticleoligonustectide conjugates. The first such method comprises binding oligonucleotides to charged nanoparticles to produce stable nanoparticleoliguanticotide conjugates. To do so, oligonacleotides having covalently bound 20 thereto a moiety comprising a functional group which can bind to the nanoparticles are contacted with the nanoparticles in water for a time sufficient to allow at least some of the oligonuclectides to bind to the nanoparticles by means of the functional groups. Next, at least one salt is added to the water to form a salt solution. The senie strength of the salt solution must be sufficient to overcome at least partially the 25 electrostatio repulsion of the oligonucleotides from each other and, either the electrostatic attraction of the negatively-charged oligonocleosides for positivelycharged nanoparticles or the electrostatic repulsion of the negatively-charged oligonucleorides from negatively-charged nanopurticles. After adding the salt, the oligonacieolides and paraparticles are incubated in the salt solution for an additional 30 period of time sufficient to allow sufficient additional oligonacleotides to bind to the nanoparticles to produce the stable nanoparticle-oligonacleotide conjugates. The

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invention also includes the stable nanoparticle-oligonucleotide conjugates, methods of using the conjugates to detect and separate muticle solds, kits comprising the conjugates, methods of numelabrication using the conjugates, and nanomaterials and connecticutions comprising the conjugates.

- The Invention provides another method of binding oligouscleodots to nanoparticles to produce anterpetricle-oligouscleodots conjugates. The method comprises providing oligouscleodots, the oligouscleodots comprising a type of recognition oligouscleodots and a type of diseast oligouscleodots. The oligouscleodots and the nanoparticles are contacted under conditions effective to allogouscleodots and the nanoparticles are contacted under conditions effective to
- 10 silver at least some of each of the types of oligoratelectifics to hind to the necessariation produces the conjugates. The invention tool includes the annequation disjunctional reconjugates produces by the month, melado of silver the reconjugates to desire and requires souther soid, have comprising the conjugates, methods of searchitections can give conjugates, melados classifications and considerations and conjugates. The comprising the conjugates and associated in an descriptoristic search search of the conjugates. The comprising the conjugates and associated in an advanteration and search of search conjugates and produces of parameteristics are oligoratedosteristics. The superior of a market is side of oligonatelectificity and its less produce of the superior of a market is side of oligonatelectificity and less that the produce of the conjugate of the comprision of comprehendations are superior of the conjugate of the conjugate of the comprehendation of the comprehendation of the conjugate of the comprehendation of the comprehendation
- 20 The invention provides yet another method of briding oligonateoides to anospiral on in produce managemide-dispassionides comprising sortification and comprising sortification of the comprising sortification of the comprising sortification oligonateoides. The recognition oligonateoides comprising a menographic perfine and a sporae portione. The recognition pursion of the recognition oligonateoides comprising oligonateoides the size required comprehension of the sortification oligonateoides have a regiment occupatementary to at heat on portion of the

to be bound to the nansperticles or to bind to their targets.

- 25 oilgonostestides has a sequence comprementary in it needs only person or task sequence of a mustic said or disponse-bedied target. The inputer person of the recognition oilgonucleoside is designed so that it can bind to the amazonateles. As a result of the infinite of the spacer portion of the recognition oilgonucleoside to the managonateles, the recognition personal is spaced very from the surface of the
- 30 mesopurioles and is more secresible for hybridization with its target. To make the conjugates, the object-valentieles, and the recognition objects of the object and the conjugates.

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nanoparticles use connected under conditions officetive allow at least some of the recognition oflipstancherolists to blad to the antemportricles. The interiorism date tractional this maneportricle oflipstancherolists organized produced by this method, metabols of using the conjugate to detect and experient models cacle, both comprising the conjugates, methods on from Obstication using the evolutions, and summerical and

amontures comprising the contiguence.

The inventors comprises a method of threships of aground rollers to empregation by genue of a labor comprises a verbal of the shades experiment of a labor comprises a verbal collect in shades experiment of a labor comprises a verbal collect in shades experiment of a labor comprises a verbal collection of the confider control and a variable to ever shades a verbal collection of the confider control as a variable to every district for the confider and an advantage of the confider control and experiment of the confider control and experiment of the confider and an advantage of the confider and and a verbal collection of the confider and an advantage of the confider and and advantage of the confider and an advantage of the confider and an advantage of the confider and an advantage of the confider and advantage of the confider and an advantage of the confider and advantage of the confider and advantage of the confider and and advantage of the confider and advantage of the confider and an advantage of the confider and advantage of the confi

unoposition as heart had bearing. A transportation of the a plurality of oligomolecular microscopies, a "type of disposable olicita" refers to a plurality of oligomolecular microscopies, bearing a transportation, "the "transportation," the production of the production of the standard fluctuation of the standard production of the standard fluctuation of the standard fluctuation, "transportation objects are plantared for the standard fluctuation," transportation of products," assumption of the standard fluctuation of the standar

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# BRIFF DESCRIPTION OF THE DRAWINGS

Eignet 2: Schemate diagnam libertaining the financian of tumopraticle aggregates by combining numperational training complementary oriignmenteroides attacked to these, the manaparelistic bring half highest in the aggregates as a result of the hybridization of this complementary originationics. Nemperature any conviction method (units in SCH5):pD(PO(SO), when Re is joined as paid assuspentiols. For

### WO 01/05/665

PCT/LS01/01190

the sake of simplicity in Figure 1 and some subsequent figures, only one oligonatelosticle is shown to be matched to each particle but. It fact, each particle has several oligonatelosis satisfact for at Albo, it is important notice that is Figure 1 and subsequent figures, the relative sizes of the gold assopanticles and the

5 oligenucleotides are ant drawn to scale.

Figure 2: Software diagram liberating a system for describing nucleic sold using a loxogariticito having oligomethoridan attached thereto. The oligomethoridars on the two anapparticits have requirence complementary to two different portions of the independent of the complete and producing a contract of the contraction of the described having (froming aggregation and producing a color change).

Example: Showasted ingigen of a varieties of the system shows in Figure 2.

Be alignated interes in the her homographic how sequence conjecturity in two difference partices of the single sextended DNA shows which are separated by a whole perform which in the complementary to the conjecturity and in subjectived which are sequented by a which perform which in complementary the subjectived byte of the empower of the subjective state of the subjective sequence of the subjective state of the subjective subjective state of the DNA, who conjectured the subject of the DNA, necessariate and filter disponsedurated are constituted to the suspensation of the subjective state of the subjective state of the DNA, necessariate and filter disponsedurated are constituted to the suspensation state of the subjective state of the s

Figure, 4: Schematic diagram filtutation reversible aggregation of 2 management of the production of the statement of the result of Tayloridization and de-hydridization with at liaking diagrameteride. The librarized limbing oligonatesistic is a doubte-attended DNA having overlanging terminal (felley works) which are complementary to the objector/confidence and the first approximation of the composition of the statement of the composition of t

Figure 2: Sobrmatic diagram illustrating the formation of nanoparticle
25 aggregates by combining nanoparticles having eligentacionides attached thereto with
linking nilgeometeodides having exquences complementary to the olignouriseoxides
attached to the nanoconticles.

Figure fr. Curetten containing two types of gold colleids, each having a different oligonatestated antached thereto and a linking double-formed 30 oligonatestated with stickty cash complementary to the oligonatestated attached to the nanoparticities (see Figure 4). Curvate A - at 80°C, which is show the Tan of the

PC1/4/501/01199

result in A

Biolog DNA, de-Apiedicas (betwandly denomend). The other in derive mt. Currens 8

- after cooling to room temperature, which is below the Tim of the Histong DNA;
hybridization has taken joice, and the inneparative have aggregated, but the
aggregates have not provipitated. The other is purple. Current C - after several hours
5 at room temperature, the aggregated annoparativists have seetfiel to the bottom of the
current. The solution is clear, and the provipitate is jorishing by: Mexing Bor C will.

Figure 2: A graph of absorbance versus wavelength in an showing changes in absorbance when gold annoparticles having oligonucleotides attached thereto

aggregate due to hybridization with linking oligonucleotides upon lowering of the

aggregate due to hybridization with limiting ollipseucleotides upon lowering of the temporature, as international in Paper. A <u>Eligente SA-IE</u>: Figure SA is a purple of change in absorbance versus temperature/limit for the system illustrated in Figure 4. At low temperatures, gold managraticles having eligenucleotides entsched thereto aggregate due to hybridization.

15 with Intology oligomenteration (see Figure 9. A) high Interpretation (40°C), the managerations are die hybridested. Changing the immorpation over time thouse that this is a reversible process. Figure 88 is a period change in the otherwise series temperature/time performed in the same manner using an approas solution of unemailliog gold unequanticles. The reversible changes seen in Figure 8A are not observed.

Figure, 2A-32: Transmission Electron Microscope (TEAs) Images. Figure 9A in a TEAs image of aggregated gold anapparities held together by bybesitization of the objectation on the pold anapparities with linked optional-toolsides. Figure 92 is a TEAS finange of a two-dimensional suggregate aboving the ordering of due to the transmission of the pold of th

Figure 10: Solumethic diagram illistrating the formation of thermally-stable triple-transfel oligonactionide connections between managements having the pyrimidilar purposphelidian most 10-short-tiple-canadactions are slifter than double-manded connectors. In Figure 10, one management has an oligonacticotide stable to it which is composed of all parisines, and the other transportation has an oligonacticotide adjustment of the other transportation has an oligonacticotide adjustment to the whole transportation has an oligonacticotide adjustment to the other transportation has an oligonactic oligonactic ordination. The third

WO 01/05/645

PCT/USOLOUI/0

oligonucleutide for finning the triple-stranded connector (not attached to a nanoparticle) is composed of pyrimidines.

Ejezz, 1.1: Schemowie die Agene Illustrating de formation of anosperation aggregates by combinities consequentiales being necessitated in compositated being anosperation being necessitated being manufacture of the compositated being head to agente in the aggregates we a result of the hydrodization of the compositated production of the compositated compositated or the compositated compo

10 Einzet 12A-E: Schemate diagrams illustrating synoms for descring nutriciand diagrams and sing samparticles having disgonatedosites natured after configuratedoside management configurate and 2 and single-stranded oligonatedosites trapets 3, 4, 5, and 7 are illustrated. The circles represent the management configurated supersection of the description of the description.

<u>Figures 13A-B</u>: Schematic diagrams illustrating systems for detecting DNA (mailyte DNA) using nanoparticles and a troupscent substrate.

Estates 14-16. If Figure 14-16 is a gray of all shorteness versus severdage, it may devote change in the herchenes where gold monoposition having algorithmetical 20 anished transce (see population of which is in salarion and one population of which is standard to its resupposes solvention as illustrated in Figure 1309; aggregate due to hapitudization in history eloppementation. Figure 1401 appel of offence in shortforce for the hybridization shorteness for the hybridization shorteness for the hybridization shorteness.

5 Excess 13.6.C: Schoeste diegrems illustrating systems for detecting modelic-acid uning manquericien having oligoausteocioides antanche herei. Oligoausteocioides manquericie corigipates 1 and 2 and single-stranded oligoauschruiche tergets 3, 4, 5, 6, 7 and 8 are illustrated. The circles respects the manquericiet, the formulas are oligoausteological exposurce, and 5 respects the thin-baskly Maker.

O <u>Figures 16A-C</u>: Schematic diagrams illustrating systems, for detecting modele acid using nanoperticles having oligometeotides attached thereto. Oligometeotide-

W-22 281 01 W

nanoparticle conjugates 1 and 2, single-attracted oligonociestics targets of different lengths, und filter oligonociosides of different lengths are illustrated. The careles represent the nanoparticles, the formulas are oligonocioside sequences, and S represents the bid-sibly limiter.

3 Excess 12A.E: Schematic diagrams illustrating nanoparticle oliposmionististic conjugazes and systems for denesting markets sold using unspectivelts having objectuations startands dentest. The sold represent of nanoparticle having interseprent oligosuscionistis extended dentest. The sold represent of supposudoristic desiane deates not sharely, two oldersty-special possibility interseprent oligosuscionistic desiane deates not sharely, two oldersty-special possibility and produced participates and sold produced produced

10 seguente (ia is comprenentary as a pro somprenentary our configures 25 Schematic disagram Materialize a system Coderologia medici acid uning lijosomes (terga double circle), hanoparticles (small open circles) and a temperate substrate. The fillicl-in squares represent clockettery) groups, the spingles represent cligatoric choice, and the indees represent double-standed (hybridized) of agreement cligatoric choice.

Times 19.4.1: Figure 19.4 is a graph of shootheave versus wavelength in term shoring thangs in shootheave when gold nanoparticle-oligened solds congusted assemble in multiple layers on a transparent substrate as liturated in Figure 13.4. Specially 19.5 is a graph of change in shootheave for the hybridized system referred to in Figure 19.4 as the temperature is increased (mistrics).

<u>Figures, 20A-B</u>: Wasterdone of actemes using fluorescent-labeled dignusclossides attached to mobilite or scusconductor quencing comparticles (Figure 20A) or to non-metallic, non-semiconductor particles (Figure 20B).

Figure 2.1: Schemiste diagram libratrining a system for decening trept
motive and using upon emospherites barrian of general color attacked there are
blace nitrosphares having finerecentrally-libride oligamorization standard disease.
The small, obsect dark circles represent the nanoparticles, the large open circles
represents the term emicroplares, and he large over represents a seriespecture.

10 Figure 22: Schematic diagram illustrating a system for detecting target made a said using two types of thorousently labeled eligonacteotide-manaparticle

# WO 01/05/1665

# PCT/ASDLO1190

conjugates. The closed circles represent the nanoparticles, and the large oval represents a micropropos membrane.

Figure 23: Sequences of materials utilized in an assay for Amthrax Protective Artigen (see Example 12).

5 Tions 26. Schemusic diagram illusirating a system for detenting target matrics intil using a "statellite protor" which comprises majorate unsequenched (article protor) which comprises the unsequenched (article protor) in the contract of the contract of

with A, R and C being complementary to A', D' and C', respectively.

Figures 25.0.1: Schematic diagrams illustrating systems for detecting DNA
using nanoparticles and a transparent substrate. In those Spares, a, b and c refet to
different objectoric requirement, and a', b' und c' effect to objectoric objectoric detection.

15 sociationes complementary to a, b and c, respectively.
Figure 26: Schematic diagram illustrating systems for forming assemblies of

CoStruit General and quantum done (QO).

Figure 22-20. Figure 27.4 downs Elementone spectra comparing dispared and agragated (Dr.), with an excitation at 600 att. The surplets were proposed identicable, second for the addition of complementary fisher? UMA to be obtained in electricable, second for the addition of complementary fisher? UMA to be obtained from concentration of new exceptions surplet first the source of ground concentrations of new exceptions surplet first the special configuration. That is may be a partition of a physical polyCOD assorbly. The intellection fixes of the ODs. which are second polycologist complete in the first polycologist COD, which can be also supported to the contract of the c

experiments were conducted in 0.3 M NaCl, 10 mM phosphare buffer (9H 7), 0.01 %
solium arisle with 13 mm gold unapparticle isolder =4 mm CASM/7AM contribled QTm.

27

PC1/4301/01190

Figure 28A-E: Schematic diagrams illustrating the preparation of core probes, aggregate probes and systems for detecting DNA using these probes. In these figures, a, b, c and d refer to different alignment of the production of the defect of

Figure 22: Graph of fractional displacement of oligonucleotides by metaphoethanol from nanoparticles (closed circles) or gold thin films (open squares) to which the oligonucleotides had been structed.

Figure 19: Graph of surface coverages of recognition oligonucleotides on nanoparticles obtained for different ratios of recognitionalihems oligonucleotides to used in the proparation of the nanopasticle oligonucleotide conjugates.

Figure 31: Graph of surface coverages of hybridized complementary oligocuclocides versus different surface coverages of recognition oligonsolectides on narroparticles.

Figure 32: Schematic diagram illustrating system for detecting a larged DNA

15 in a four-element array on a substrate using nanoparticle-oligosucleoside conjugates
and amplification with silver staining.

The properties of the time of the properties of

Figure 33: Graph of groyscale (optical density) of aligorateleotidefunctionalized glazs surface exposed to varying concentrations of target DNA.

WO 01/051665

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followed by 5  $_{12}$ M gold of nanoparticle-oligonnoleonide indicator conjugates and silver amplification for 5 minutes.

Figures 35A-B. Graphs of percent hybridized label versus temperatures and percent hybridized label versus temperatures. showing dissociation of fluorophore-labeled (Figure 35A) and nanoparticle-labeled 5 (Figure 358) jurgets from an oligouscleotide-functionalized glass surface. Measurements were made by measuring fluorescence (Figure 35A) or absorbance (Figure 35B) of dissociated label in the solution above the glass surface. The lines labeled "b" show the dissociation curves for perfectly matched obgomulatedes on the glass, and the lines labeled "Y" show ourses for mismatched oligonucleotides (a 10 ane-base mismatch) on the glass. Vertical lines in the graphs illustrate the fraction of sarget dissociated at a given temperature (halfway between the melting temperatures  $T_{\rm sc}$  of each curve) for each measurement, and the expected selectivity of sequence identification for fluorophere- and nanoparticle-based gene chips. Fluorescence (Pisone 35A): complement (69%)/mismatch (38%) = 1.8:1 Absorbance (Figure 15 35B): complement (65%)/mismatch (14%) = 6:1. The treadth of the floorophore labeled curves (Figure 35A) is characteristic of the dissociation of (luonsphore-labeled targets from gene chips (Forman et al., in Moloculur Modelling of Nucleic Acids, Leontis et al., eds., (ACS Symposium Series 682, American Chemical Society.

Wendangson D. C., 1998, pages APO 229).

\*\*Britter Mach. The trust of model disjunctionists array challwaged with synthetic target and flavoresm-bidded (Figure 244) or trustgenization behald (Figure 234) or trustgenization behald (Figure 244) or trustg

Figure 17: Schemele diagram illustrating system for forming aggregates (A) or layers (B) of nanopartitles (a and b) hinted by a linking nucleic sciel (B).

10. Pigure 18A: UV-visible upcoten of differenting leyers of gold nanoparticles a and 6 (see Figure 37) hybridized to un oliquenticonde functionalized glass.

### WD 01051665

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microscope slide van the complementary linker 3. The spectra are for assemblies with 1 (a,  $\lambda_{mn}$  = 524 nm), 2 (b,  $\lambda_{mn}$  = 529 nm), 3 (c,  $\lambda_{mn}$  = 532 nm), 4 (d,  $\lambda_{mn}$  = 534 nm) as 5 (e,  $\lambda_{mn}$  = 534 nm) layers. These spectra were recassred directly through the

- 5 Figure 38B: Graph of absorbance for nanoparticle assemblies (see Figure 38A) at \(\lambda\_{\text{min}}\) with increasing numbers of layers.
- Figures 39A-F: Figure 39A: FE-SEbt of one layer of oligonucleotidefunctionalized gold nanoparticles cohybridized with DNA linker to an
- oligonatestide-fluedismitted, conductive indirect-fluedistic (ETTO) alloi (prepared in the stras way as oligonatesticile-fluenationalized plass siled;). The visible absorbance spectrum or this side: wie identical to Figure 38A, inclinating that fluorionalization and anraparticle converge on ITO is sirealize to that on glass. The average density of counsed annaporticile from 10 south images was approximately \$800.
- managuriclesium<sup>3</sup>. Figure 1988. FE-SEM image of two layers of managaridate on 15 the TTO 1640. The everage density of countried interpretations from 10 such images v.33 expressionates/250 particlesium<sup>3</sup>. Figure 399. CS. Alsachenae 1020 sun (A.vaga) showing dissociation of a 0.5 pM solution of the obligament established draptics (1 = 2 + 3; see Figure 37). At to single errors in 0.3 M No.C, 10 mMy phosphate briffer solution (6M 1.7). Hencey 3500-PS. Alsachenae or 30 pm (A.4) and possible situation solution.
- 20 Figure 30(), A layer (Figure 30()) and 10 layers (Figure 30()) of disposatedwide-functionalized gold nanopartics from glass alides interested in 0.3 M NaCQ, 10 and phosphate buffer solution. Meeting profiles were obtained by measuring that decreasing chargeful and 250 use (Anga) through the alides with increasing temperature. In each of Figures 1907-1, the loasted twen that fact derivatives of the
- 25 manuted disociation covers. FWIIM of finises cureou wee (Figure 296 Cases) 33.2 "C. (Figure 290 Inter) 3.6 "C. (Figure 398 Inter) 3.2 "C., and (Figure 299 Finars) 2.9 "C. [Higgs: 45]: Schemadic diagram literateding system used to measure the electrical properties of gold annoquarities assembles Indeed by DNA. For simplicity, only one hybridization event is design."
- 30 Figure 41: Schematic diagram illustrating a method of detorning nucleic acid using gold electrodes and gold ranoparticles.

PC174301/01190

Figure 2: Vacamusis changes is Blanching the environment of a cyclic clustified. In multi-ling dispersion of the cyclic clustified. In multi-ling dispersion clusters, a transfer distribution to managericles. The second distribution materials was obtained by conductation of 4.5-ship/devs.). 2-challens with gashed second confidence in confidence was or growed and gibesestories the modified with the steroid distribution chairs greater materials where the product of the confidence of the confidenc

preparation.

<u>Figure 43</u>: Schematic diagram for the synthesis and formules for the steroid

10 cyclic disulfate auchor group.
Figure 44: Schematic diagram illustrating cyclic disulfides of forentals 2 tor use in preparing oligocuschetido-cyclic disulfide linican as described in Example 24, and same related cyclic disulfides for use on another groups.

Figure 45: Schematic diagram illustrating the structures described in Example

2.5. Figure 45(4) Illustrates the structurers of 5'-monochitid-modified obligomostoxide 5, a 35-base 5'-more old distalled obligomer 6 and Tembler phosphoromide 7 and 5-fri-more oppositively obligomechanide 8.

Figure 46: Schematic diagram illustrating the chemistry of making a novel trithiol oligomolectics. WD 01/051/65

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DETAILED DESCRIPTION OF THE PRESENTLY PREFERRED EMBODIMENTS

Nexopartics authit in the protect of the treatment include actual (e.g., paid, blue, coppered and plustions), estimated and five for the control include actual (e.g., paid, blue, coppered and plustions), estimated actual (e.g., Calles, C.U.); and C.S. of C.U.S. of C.S. of C.U.S. of the comparation of the control include 25, d. of C.O. of C.D. of J. of A. of C.O. o

Methodo of realizing meals, senzionoductor and negapotic susopasticles are subsultikatoro in fine ni. Son. q., Schotter, (d. Q.C.) Canter and Goldon (CVL),
Weitzichm, 1990), Huyut, M.A. (ed.) Cultostad Gold: Principlex, Methodo. and
Methodon (Collection (Ancientic Press, San Bage, 1991)), Hoston, R., 1992. Teamusestime

15. On Judgameter, 1, 1207 (1991), Amerika, S. et al., Schotter, 27, 1924 (1996);
Henglin, A. et al., J. Phys. Chem., 99, 14129 (1995); Gurits, A.C., et al., Aegono.
Chem. In. Ed. B. (20, 27, 1936) (1998).

25 Unida et al., A Phys. Chem. 39, 3544 (1992).
Saitable nonopariedas sea inno constructivity available from, a.g., Ted Polh, inc. (pold), Americaham Componitorida (pold) and Namportock, Inc. (pold).
Percently preferred for sea in denoting marine scales are guid manoparicient.
God colloidad practice have high audientien conflictions for the bands that give rior to the her bands for continuous conflictions.
God colloidad practice have high audientien conflictions for the bands that give rior to the branch for the properties of the continuous conti

### WD 01/051665

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of the aggregates, making these materials particularly attractive for colorimetric usays. For instance, hybridization of eigenvalentides stached to gold nanoparticles with oligonoclouides and succiois acids coults in an immediate color change visible to the naked eye (see, e.g., the Examples).

- Gold autoparticles are also presently preferred for use in autophthication for the same routine; given above and beauss of their stability, ease of imaging by decrean misconcey, and well-characterized modification with shield standardise (see bolow). Also preferred for use in macelifacturion are semi-condector autoparticles between of their unique electronic and functioned preparties.
- The parceparticles, the ofigonucleotides or both are functionalized in order to airculus de oligorusticodises to the unequarticles. Such methods are lazown in the art. For instance, disgonucleotides functionalized with all Section list as their 3-terminal or 5-terminal readily states to gold associations, See Whiteides, Proceedings of the Robert J. Revision Foundation 30th Conference Co. Consect Research Hemographics
- 15 Chemistry, Houston, TX, pages 109-121 (1995). See also, but-ic et al. Chem. Centron... 555-557 (1996) descorbes a method of muching 3' mid IDNA to East gold surfaces; his method can be used to attach oligorationslets to amountation.' The althoredized muchou can be used to attach oligorationslets to the method can be used to attach oligorationslets to other metal, suntinovalutors and imagnifice collision and to the other accordance of the metal.
- 20 Other functional groups for attaching oligomethesistics to solid armfares included phosphoenidesist groups (see, e.g., U.S. Patert No. 3,477,381 for the bindings of eligomethodistic hephosphoenidesiste and goal surfaces), substituted sklyphilassanes (e.g., Birrord, Chemical Tzechnologo, 4, 370-377 (1993) and Matinacel and Greathens, Chemical Tzechnologo, 4, 370-377 (1993) and Matinacel and Greathens (e.g., Birrord, Chemical Tzechnologo, 4, 370-377 (1993) and Matinacel and Greathens (e.g., Birrord, Chemical Tzechnologo, 4, 370-377 (1993) and birth production of the control of the
- assinceably/tolinoxance and for similar binding of mercaptosidylationanes).

  Oligonateleotides terminated wish a 5' thiometeoside or a 3' thiometeoside many also be used for attaching oligonateleotides to solid surfaces. The following references describe other methods which may be comployed to statistical oligonateleotidies to
- 30 nanoparticles: Nuzzo et al., J. Am. Chon. Soc., 109, 2358 (1987) (disulfides on gold);
  Allina and Nuzzo, Longmuir, 1, 45 (1985) (carborytic acids on obscessionin); Allara

PCT/ES01/01190

and Tompsins, J. Collect Analysis Adv., 41 (1-41) (1975) (carboxylic unide) on capped, its pt. Experimer (25 like), Desiry (New (1979) 1979) (carboxylic unide) on site, Timmuns and Zimmu, J. Phys. Chem., 95, 944-950) (1950) (carboxylic unide) on philomy), Frincipa and Inhabeed, J. Ace Chem., 50, 193, 1971 (1970) (consolicit on philomy), Frincipa and Inhabeed, J. Ace Chem., 50, 13, 177 (1970) (consolicit on subhidities and object in action and the site on philomy). Hollows and a site, J. Am. Chem., 500, 1117, 1771 (1989) (considient on philomy). Hollows and site, J. Am. Chem., 500, 1117, 1771 (1989) (considient on philomy). Hollows and site, J. Am. Chem., 500, 1117, 1771 (1989) (considient on philomy). Hollows and Silvey (Anappents, 3, 1984), Anappents, 1304 (1989) (dimiters on callialy). Moreover flows), Anappents on charge (1987) (silvey), Anappents, 3, 1984 (1987) (considered exceptly circle), distribute, Analonius on matheway groups on inharism dentities and alles), Lies et al., J. Phys. Chem., 92, 3297 (1997) (silvey) consortium).

Oligonucleoides functionalized with a cyclic distalfide are within the scope of the invention. The cyclic distalfide preferably have 5 or 6 atoms in their rings, including the two suffer atoms. Sainable cyclic distilfides are available commonically or may be symbosized by known procedures. The roduced form of the cyclic

or may be synthesized by known procedures. The reduced form of the cyclic distillides on also be used.

Particaciós, cha fisicia fendere comprises a hydrocontron moticiy statedels to be cyclic citatidis. Southie hydrocottes are countiles commercialis; and se stateded to the cyclic citatidis are principally the hydrocottes moticiy is a surred resident. Oligosuchocidos moticiny properties impligates reconfigurate prostage in sergio descripation and survival and analysis to a cyclic distuitation have unexposently bene flourid to be remarkably saide to this vide, of admithenties have the exposent solit; protection CEU solitations as comproperte loss places flouride protection descripation in cyclic distuitation as the control of the control of the comproperte loss places flouride in cyclic distuitation as the control of the comproperte loss places flouride in cyclic distuitation and the control of the comproperties of the control of the comproperties of the control of the comproperties of the cyclic distuitation and the control of the comproperties of the cyclic distuitation and the control of the comproperties of the cyclic distuitation and the control of the comproperties of the cyclic distuitation and the control of the comproperties of the cyclic distuitation and the cyclic distuitation an

30 advantageous in stabilizing the oligonacteotide nanoparticle conjugates. The large hydrophobic steroid residues of the linkers also appear to contribute to the stability of

WO 81/051645

PCT/LSOL01190

the conjugates by acroening the nanoparticles from the approach of water-subble molecules to the surfaces of the nanoparticles.

In view of the finespring, the two unifar atoms of the cyclic distulfed should preferredly be close enough together to this both of the sulfer atoms can attach 5 minutizationarily to the amosparation. Most preferredly, the two sulfer atoms are adjacent such where. Also, the hydrocarbon mointy found be large to as to potent a large by jobipolish sourfice according the unifers of the amosparaticles. The deglacenticitation cyclic management that traplety cyclic.

The oligomotive date specific insequential environment that croping specific distribution in the specific ways the seal of probes in deprotein easiety for deterting souther than other than the specific contribution of the cont

The suppliest grantifies of the senting, adjustment-order more particles or originate for the version to thick gloring and the real-low them to be call discretify the control of the cont

Finally, the invention provides kits comprising a container hobiting a type of obligament-notice cyclic distultible insteas of the invention or a container hobiting a type of obligament-notice-manipurative negligation of the invention. The kits may also contain other resignets and items useful for detecting models acids or for manifoldimically.

### PC'112S01/01190

Each nenoparticle will have a plurality of oligonucleotides attached to it. As a result, each nanoparticle-oligonucleotide conjugate can blind to a plurality of oligonucleotide on nucleic acids having the complementary sequence.

Diligense-bosition of risknet sequences are used for a variety of progress in the practice of the invention. Methods of making olgonocismines or a professmined sequence as var dishroom. See, e.g., Sambrook et al., Moderade Comang. A Jahro story. Manual Clark et al. 1999 and F. Escharine (ed.) Olgonocismines and Analogous, 1.16 Colford University Proc. New York, 1991). Solid-place synthesis methods are professed for both oligoribonocitosiste and

10 oligodroxyribonucleotides (the well-known methods of synthesizing DNA are also useful for synthesizing RNA). Oligombonucleotides and oligodroxyribonucleotides can also be prepared enzynatically.

The Invention provides methods of detecting zucleic saids. Any type of nucleic acid may be detected, and the methods may be used, e.g., the the diagnosis of 15 disease and in sequencing of nucleic acids. Examples of nucleic acids that can be detected by the methods of the invention include genes (e.g., a gene associated with a particular disease), viral RNA and DNA, bacterial DNA, fungal DNA, cDNA, mRNA, RNA and DNA fragments, oligonocleolides, synthetic oligonocleolides, modified offgonucleotides, single-stranded well-fundle-stranded nucleic soids, natural 20 and symbolic medica acids, etc. Thus, examples of the uses of the methods of detecting puckeic acids include: the diagnosis and/or mentioning of viral diseases (e.g., human immunodeficiency virus, hepatitis viruses, herpes viruses, cytomegalovirus, and Epstein-Barr virus), bacterial diseases (e.g., toberculosis, Lymr disease, H. pylori, Richerichia cali infections, Legionalla infections, Mycaphasma 25 infections, Solvenella infections), sexually transmitted diseases (e.g., generable). inherited disorders (e.g., cyalic fibrosis, Ducheus muscular dystrophy. phenylketonaria, sickle cell enemia), and cancers (e.g., genes associated with the development of cancer); in forcesies, in DNA sequencing; for paternity testing; for cell line authentication; for monitoring gene therapy; and for many other purposes. The methods of detecting medic acids based on observing a color change

with the naked eye are cheap, fast, simple, robust (the reagents are snable), do not 36 PATTA SALIO 190

require specialized or capenaive equipment, and little or no instrumentation is required. This makes them periodetely softlible for usin, me, as recented and enablytical bioperatories in DFA sequencing, in the field to dolect the presence of specific pathogens, in the doctor's office for quick identification of an infection of saids in prescribing a dought or summent, and in hones and health centered for

 assist in prescribing a drug for measurent, and in homes and leadth centers for inexponsive first-line successing.

The markies and to the destined may be soluted by Jacoms methods, or may be destined directly in the first, firstess mergin, belongiated that (e.g., a though mine, then (bed, a mengin, sholidess conticiting ECR acceptances, solutions corrating large consensed of algonate-conforce with phin methods: well global. Not, not of our rampher, as and be above in the mile. 1999 and ED, Backers and EJ, Highers and EJ, Care Polives I (AP). Now, Now York, 1993). Methods of proprinting senders under the interface of the senders of th

If a nucleic acid is present in small amounts, it may be applied by methods known in the act. See, e.g., Sambook at al., Moleculor Cloring: A Laboratory Monutel (2nd ed. 1989) and B. D. Hames and S. J. Higgins, Bed., Guer Perbed r (RL)

Press, New York, 1999). Preferred is polymenus etchic reaction (PCR) amplification.

Des method exceeding so the in-westion for describing under and demonstrate contenting a method and side done on error topic of computation for large and grant gr

Also, when a nucleic sold is to be detected in the presence of other nucleic acids, the portions of the methic sold to which the oligoundemirles on the

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nanoparticles are to bind must be chosen so that they contain sufficient unique sequence so that detection of the machic acid will be specific. Guidelanes for doing so are well known in the art.

- Although matelia acids may contain reproduce parquetter close enough to each other city on type of oligenedeval-manupartial conjugate read to med. this will be an excurrence. In general, the chosen proteins of the matelia cald will have different cognitions and will be constanted with annoyamidate carrying two or new different origeneouslooks, perferrably stended to different manuparticles. An example of a system for the detection of medica add is situated and fifteen or figure 2. A case of the company of the system of the detection of medica add is situated and figure 2. A case of the company of the system of the detection of medica add is situated and figure 2. A case of the company o
- 10 he seen, a first oligomacioside attached to a first nacoparticle fina a sequence complementary to a first porcino of the target sequence in the single-stranded DNA. A second oligonocioside attached to a second misoparticle fast a response complementary to a second purificion of the target stagement in the DNA. Additional portions of the UNA could be transplant with corresponding nanoparticles. See Figur
- portions of the DNA could be targeted with corresponding nanoparticles. See Figure
  15 17. Targeting several portions of a nucleic acid increases the magnitude of the
  detectable change.

The contacting of the mempurische-oliginant-studies configures with the number and takes pitter under conditions effective for hydroidization of the oliginanclerisation on the monoprische with the target expenses(e) of the president axis. These by hydridization conditions are well known in the set and can readily be oplicated for the precisular system supplyed. See, e.g., Sunbrook et al., Molecular Climing: A Lebomory AbunQuil Carl et 31989. Preferribly stringent by decidization conditions as

employed.

Fasts hybridization can be detained by freering and thewing a solution

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muctoic unids. The hybridization is complete, and the detectable change may be observed, after thereing the solution.

The rate of hybridization can also be intreased by warning the substance containing the mobile sold to be denoted and the manuparticle-oligonactostide 5 consignates to a furnificant the object destroyed and the manuparticle based on the complex feet and presentative before the discontinual magnetization and the control of the complex feet and the target markets and Alexandrety, spid hybridization can be substant to have been placed as for discontinual control of the complex place from and belowing the obligation to cond.

The rate of hybridization can also be increased by increasing the sult 10 concentration (e.g., from 0.1 M to 0.3 M NeCl).

The descensible change the excess upon hybridization of the objectnedication on the managements to the market said may be a color change, the floration of an aggregate of the managements of the management with the change of the aggregate of the managements of the aggregate and perform of the aggregate of the assequents are the observed with the model or or a spectroscopically. The formation of aggregate of the assequents can be observed by determ misracropy or by supplications. The prespication of the aggregate and an activated that can be observed by desired with the model or you misconceptually. Preformed or a single solventhin with the nature of the aggregate activation of the object of the aggregate activation of the

The characteristics of a cost change with the stacked cycle can be made mean resultly against the registered and a consentation can be found to the control of the control

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stable and do not elauge on subsequent cooling or heating or over time. They provide a convenient permanent record of the test. No other stops (such as a separation of hybridized and unhybridized puroparticle-oligonucleotide conjugates) are recessary an observe the color change.

5 An Internate method for easily visualitizing the away results is to price a sumple of anapoparticle probe hybridized to a target nuclein acid ma a giase fisher filter (e.g., Marcollicate Microfilter Filter, fit a "mission pore size, grafted FOT5, for see with good nanoparticles to see in its acid, white dearwing the legical through the filter. Subsequent risining, with water wastes the excess, non-hybridized produce through the filter.
10 Leaving-behald an observable spet comprising the aggregates generated by

hybridization of the nanoparticle probes with the target nucleic sold (resined because these aggregates are larger than the pores of the tiltel). This technique easy provide for greater sensitivity, since we access of nanoparticle probes on the used Unfortunately, the amoparticle probes stick to many other solid serfaces that have

Unicormately, the nonoparticle probes stick to many other solid surfaces that have been tried (silica ables, reverse phase plates, and nylos, nitrocelluloss, celluloss and other membranes), and these parfects cannot be used.

As lagostras rapor of not democina system (Illustrated in Figure 3) is data chairing, as determined changed speakes not expectately object states of the different collegement of the control of the cont

The target sequence of the tracelete solid may be contiguous, as in Figure 2, or the two portions of the target sequence may be separated by a third portion which is not complementary to the oligouschedides on the transporticles, as illustrated in Figure 3. In the latter case, one has the option of using a filter oligouschedide which the figure 3. In the latter case, one has the option of using a filter oligouschedide which

is then in solution and which has a sequence complementary to that of this third .

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poerion (see Pigure 3). When the filter oligonucleotade hybridizer with the third purtous of the motion and, a double-stranded reguent is created, theody shrining the average distance between the nanoparticles and, consequently, the color. The system illustrated in Figure 3 may increase the sensitivity of the detection motion.

Some embodiments of the method of detecting nucleic acid willize a substrate.

By employing a substrate, the detectable change (the signal) can be amplified and the atmitivity of the assay increased.

Any unknown can be used which allows observations of the detectable thomas. Soliable substrates (include temporare solization (e.g., soliable unknown, platfor and other projumen), useque solid anterior (e.g., which cold controlers, souls an ETC Allies plate, filter press, jie.e., filter filter, earlier and ninte membrances, system semilories, and condensing useful surferces (e.g., below-dis-orded (ETC)). The colaristics can be any shape or thickness, but pressenly will be filter and fall. Preferred are insuperate to all configured (e.g., see the decide) optical (e.g., with 100 circlester plates).

15 In one embedieren, oligomeleotides zer unterdet to fine substrate. The oligomeleotides are unterdet to fine substrate. The oligomeleotides are to be attended to the cubstrates and destroited in ..., a.g., Christoy et al., Meetick Acide Res., 24, 2013-1000 (1996); Chiosiyy et al., Meleick Acide Res., 24, 2013-1000 (1996); Chiosiyy et al., Aleicke Acide Res., 24, 2014-1000 (1996); Melline Chiosiy et al., A. Fun. Sei. Technol. A. 10, 591 (1997); and Hangare et al., PERS Lat., 134, 422 (1993).

The oligonucleoides attached to the substate have a sequence complementary to a first portion of the sequence of a succide and in the detected. The suckide said in a constanct with the abstancts ander continues effective to substancts and the substancts and the substanct of the continues of the substanct on the substanct with the suchies acid. In this summer the suchies are substancts to such that the summer the suchies are substancts of the substanct. Any submost such circle such is preferably wathed from the reductive beings and suggestance obspaced such congregates.

Next, the under collection of the substitute is constructed with a Erst type of incorporation having oligonateloides antiched thereto. The oligonateloides have a sequence composition having oligonateloides antiched thereto. The oligonateloides have a sequence composition of the collection of the nation calls, of the nation call, and 30 the contasting takes pince under considering efficiency to allow hybridization of the oligonateloides on the management with the morties does, if that senarthe the fact oligonateloides on the management with the morties does, if that senarthe the fact oligonateloides on the management with the morties does, if that senarthe the fact and the senarthe senarthe contains the senarthe senarthe contains the object of the senarthe senarthe contains the senarthe senarthe contains the oligonateloides on the management with the morties does, if that senarthe the fact that the senarthe senarthe contains the senarthe senarthe contains the object of the senarthe senarthe contains the senarthe senarthe contains the senarthe senarthe contains the senarthe senarthe contains the senarthe sen

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type of unreparticles become bound to the substrute. After the nanoparticleoligonucleotide conjugates are bound to the substrate, the substrate is washed to remove any unbound nanoparticle-oligonucleotide conjugates and macleic acid.

- The alignomolecules on the first type of inapoparticles any all have the same to sequence or easy law different sequences has bytainfine with different portions of the marchie and to be descrized. When dispensionables having different expensions are used, each nanoparticle mary have all of the different elignousientoides satisfied to be or perferribly, the different elignominoides are state-field to different amougneticles.

  Figure 17 Districts the use of inapoparticle elignomicolides originate designed for the projects designed to the complete designate districts of the projects designed to the project design of the projects designed to the project design of the projects designed to the project design of the projects designed to the project designed to the pr
- 10 hybridize to multiple portions of a cucleic sold. Alternatively, the olligonacleotides on each of the first type of nonexperticles may have a plurality of different sequences, at less time of which must hybridize with a portion of the nucleic sold to be detected (see Figure 258).
- It shall, to the first type of amongunities or ligorated receivable relocated the content and to extend on the extend type of amongunities having offigurated receivable and another three. These oligonated receivable have a requirest complementary to at least a perior on of the supermet(s) of the oligonated receivable and the surface of the supermet complementary to at least a perior on the supermet(s) of the oligonated receivable and the surface confidence affective to allow hybridizations of the oligonated receivable on the first type of fromospartices with these or to be second type of compositions. After the supermetical section for the three type of the supermetical section for the product of the supermetical section for the three types of the supermetical section for the three types of the supermetical section for the three types of the supermetical section for the types of temperature and the supermetical section for the types of the supermetical section for the supermetical sectio
- professibly washed to remove any unbound nanoparticle-oligounstcodds conjugates.

  The combination of hybridistations produces a detectable obusque. The detectable changes are the same as those described above, except that the unshipte bybridizations tenth in an amplification of the detectable change. In purficults, since
- 25 each of the first type of numparticles has auxiliarle origometocities (Passing the same or different sequences) attended to it, each of the first type of interparticle-nilgometocities conjugates on hydridic or a plumitry of his second type of managaritide-ligometocities conjugates. Also, the first type of comparticle-ligometocities conjugates. Also, the first type of comparticle-ligometocities conjugates are by the hydridized to more than one portion of the mutation.
- 30 acid to be detected. The amplification provided by the multiple hybridizations may make the change detectable for the first time or may become the magnitude of the

### WO 81/051645

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detectable change. This amplification increases the sensitivity of the assay, allowing for detection of small amounts of nucleic soid.

for detection of small amounts of nucleic acid.

If desired, additional inversiof nanoparticles can be built up by successive

additions of the first and account types of managemial—alignmelookide custinguists. In 5 this way, the sumber of managemiales immedialized per molecule of staget suchein can easily further increased with a corresponding increase in intensity of the signal. Also, instead of using first and account types of managemial-oxide configurations.

conjugates designed to inforidize to each other directly, nanoparticles bearing obgometeorides that would serve to bind the manoparticles together as a consequence of physical content of the content of

Methods of moking the nanoparticles and the olligenucleotides and of attaching the olligenucleotides to the nanoparticles are described above. The hybridization conditions are well known in the art and can be readily optimized for the perticular system complexed (see above).

5 An example of this method of descriting models acid (mmlyes DNA) is illustrated in Pigers 13A. As shown in the Pigers, the combountion of hydridizations produces dark seron where numeration aggregates are linked to the redustrate by analytic DNA. These dark uses may be readily observed with the maked specialing ambient light, petic ability virieng the substrate against a white background. A seen be

20 readily seen from Figure 13A, this method provides a means of amplifying a detectable change.

Another example of this method of detecting another acid is allostened in Figure 25B. As in the example liberated in Figure 13A, the combination of hybridizations produces derit, areas where an expertite a systemate see tasked to the substantial produces which may be observed with the nature of ex-

ho mother embediment, assequentielas are establed to the sidentene.

Nemoparticises can be alterabed to substrates as described in e.g., Grather et al., Janis 1/2

Chens 95, 73-7485 (1995); Bendell et al., J. Eleverosout, Chens., 499, 137 (1996); Bar et al., Lougeuist, 12, 172 (1996); Corive et al., J. Am., Chens. Sec., 124, 5221 (1992).

After the managemicles are sattleded to the substrated, object-sectionics, object-sectionics, object-sectionics, object-sectionics.

After the namoparticles are attached to the autorate, ongone condensate attached to the nanoparticles. This may be accomplished in the name manner

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describes shows for the structures of oligonacticuides to assoparticles in solution.
The oligonacticides attached to the nanoparticles have a sequence complementary to
a first portion of the sequence of a markets acid.

The midestrate is constanced with the materies acid under consideration affective as allow hybridization of the ollogeneuteorists until an immorpaticiate with the medicals and in this manner the materials and becomes bound to the substants. Unbound succision said is predestably wished from the substants prior to adding further nanopamicia-objectmical docuplagation.

Thus, a second type of acceptables having all generatoristic attaints dismost partial to granulation. These agencies complementary in a resould person on the sequence complementary in a resould person on the sequence of the market exist, and the models and show the third second person of the sequence of the market exist and the models and show the second person and the se

The alignoscionidate on the second typin of manaparticies may all have the same sequence or may have different sequences that hybridize with different portions of the matricle and the detailed. When disposal section having different sequences are used, each nanoparticis may have all of the different olligonacionidate attached to it or, performing, the different oligonacionidate may be attached to different manaparticis. See Figure 17.

Next, a hinding oligonucleotide having a solected accurace having at least two positions, the first percise being complementary to a least a parties of the supercess of the eligencucleotide on the second type of inamporatioles, it contacted with the record type of namporatiole-oligonucleotide complements board to the substrate under conditions effective to allow typeridization of the binding oligonucleotide to the

continuous criterios to amos reprontantantes to contante configuración de continuous criterios de amos reproporticios. In this monner, the birding oligonactoride 44

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becomes bound to the substrate. After the hinding oligomolectides are bound, unbound binding pligomolectides are washed from the substrate.

Finally, a filed type of a seasopated by buring objustment offers a stateful harmonic provided. The objustment offers a sequence componence type as sequence of a second protein of the budding eliginared colds. The amorpaticle object-colds coaligates are coastered with the budding objustment condition to both their planted coaligates are coastered with the budding objustment condition that condition of the budding objustment of the budding objustment could be advantaged as the coastered coaligate coastered to the component of the budding objustment of the budging objustment of the budding objustment of the budding objustment of the budding objustment of the budding object of the budding object of the object of the budging object of the budding object of the budding object of the budding object of the budging object of the object of the budging object of the object of the budging object of the budging object of the object of the object of the objec

The complication of hydrodistations produces a discretible change. The described changes are the sames a their controlled down, compared that the military beyindimetrous rest in an amplification of the described human. Let particular, some such of the second type of casesporticles in multiple adigramatices for priving the same of different expenses of alternative analyses of casesporticles in any interest of the same of the second type of casesporticles objected on the particular of the second or compliants of the second conjugate on the hydrodist or to provide the conception of the mortics of all to the discrete. The amplification provides the particular of provides of the mortics of the second conjugate may be hydrodist or to provide the conception of the mortics of all to the discrete. The amplification provides the described change. The amplification increases the manifold of the second control for the first free or my to increase the analytics of the described change. The amplification increases the manifold of the second control for the first free of the manifold of the described change. The amplification increases the manifold of the described change. The amplification increases the manifold of the described change. The amplification increases the manifold of the described change. The amplification increases the manifold of the described change. The amplification increases the manifold of the described change.

If desired, additional layers of amorpainithes on he balls up by successive additions of the binding oligonoclosticities and second and third types of amorparicle-oligonoclosticities conjugates. In this way, the resopratidal amorbilities are milecules of agoust motion was can be further socretod with a corresponding increase in intensity of the island.

Also, the use of the binding oligonocleotide can be oliminated, and the second and third types of managements—oligonocleotide conjugates can be designed so that they hybridize directly to each other.

Methods of making the surreparticles and the oligonarclostides and of staching the oligonactorides to the sanoparticles are described above. The hybridization

### WO 01/05/665

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conditions are well known in the art and can be readily optimized for the particular system employed (see above).

An example of this method of detecting excloic acid (not)/rs DNA) in illustrated in Figure 13B. A shown in that Figure, to combination of hydrodizacions by products of the version temperated suggestion or linked for the substrate by smilyte DNA. These died stream may be readily observed with the makes eye as described above. As can be seen from Figure 13B, this embodiment of the method of the investion credited another means of must life high described change.

Another amplification scheme employs liposumes. In this scheme,

10 oligenuticoides are attrebed to a substate. Subtatio substates are those described above, san the oligenucleoties can be attached to the substates as described above. For estates, when he substates it seek, this can be a secondaried by condensing the oligenucleotides through phospharyd or exchangite acid groups to saminosityly groups on the substates surface (for related chemistry) see (Frahy et al., And. Chem., 67, 773-784, 1995).

The oligonucleoidees stateched to the arbitrate have a sequence complementary to a first portion of this sequence of the sendain cost to be detected. The material soil is connected with the substrate under conditions effective to allow hyberitainsian of the obligonucleoides on the substrate with the rusticle axid. In this mazzers the material wait be transferred, Any understand their field is specificately wrateful acid between sound to the arbitrate. Any understand stately lead to \$100.000 to

from the cubestate before adding additional components of the system.

Next, the nucleic acid bound to the substrate is contacted with flapourness
lawing uliqueactedates amended thereon. The oligonacteorides have a requesce
complementary to a second portion of the sequence of the unable said, and the
constanting labor pulsor under countriess wifercive and stony bybriditations of the
constanting labor pulsor under countriess wifercive and stony bybriditations of

oligurus/solides on the liposomes with the ruscleic acid. In this manner the liposomes become bound to the substrate. After the liposomes are bound to the substrate, the substrate is washed to remove any unbound liposomes and multic acid.

The oligonus toutides on the Irposomes may all have the same sequence or may have different sequences that hybridize with different portions of the nucleic scid to be detected. When oligonyaleotides having different sequences are used, each

PCT/US01/01199

liposome may have all of the different oligonuclentides attached to it or the different oligonucleotides may be attached to different liposomes.

To proper edigenate-indexide-inposents conjugares, he alignmentation as televant to a hydrogenic parts on the second parts of the conjugares are second parts of the conjugares are mixed with a collection of ligenomes on the hydrogenic produced for engineers are mixed with a collection of ligenome with hydrogenic produced and produced in the members (see 22 mag et al., 7 resultable), alignomicalistic conjugares are mixed with a collection of ligenome are the mixed produced by the conjugares are mixed with a collection of ligenome are the mixed by described and produced conjugares are searched of the ligenomes can be extended by commelting the ratio of the collection of the searched of the ligenomes can be extended by the conjugares are the collection of the c

10 lydrophotic eliganot footdo ensignate se flyocomes as me missres. Il has been electred that lipozomes to braing obliganot installed as marked by lydrophotic interestation of posterio deleterary groups are effective in trapiting polymentoristics immediated on antirocullalors membrane (IA). Placesceles groups andone it in the membrane of the lipozomes were used in the property group. They served effertively, but sensitivity of the lipozome were used in the property group. They served effertively, but sensitivity and sensitivity of the cita and the signal from fluoreceives in regions of fleigh board accountanties (e.g., on the Spanesse sentition) is washaned by self-quenching

The lipsecomes are made by enthods well known in the act. See Zhang et al.,
Tearnhofoun Lett., 37, 262 (1996). The lipsecomes will generally be about 5-50 timeslarger to size Giometric libant the asseparities such in subsequent steps. No interactions
20 de nanoporticles about 1 h ms in disuntées, lipsecome about 100 un la obsesseer are
prefessibly such productions.

The lipocomes bound to the softeness are construed with a first type of emportation having at least a first type of oligomotopides statehold derects. The first type of oligomotopides statehold percent for the statehold of the conditional states at hydrochie group statehold to the end not attached to the conditional statehold the statehold of the conditional statehold cond

The method may further comprise contacting the first type of nanoparticleusgemententide conjugates bound to the Hiposumen with a scoond type of an enoparticles having oligomaleotides mustined thereto. The first type of nanoparticles have a second type of oligomaleotides attached thereto which have a sequence

PCT/US01/01190

uneplimmary in a test a position of the requests of the collispositionides on the second type of associately not ill consistent, and the obligationides on the record type of associately not ill consistent in the respective complementary is it have a position of the sequence of the sequence of the consistent on the test part associated on the test and second type of the second of the test of the obligation of the test of the obligation of the test of the obligation of the

The combination of hydridistations produces a describe chings. The described charges are the same as thor method below, the charge are the same as thor method below, nearly the the methods hydridistation tents in an implification of the ristentiate charge. In particular, steed of the lipsomen laws multiple adjustment-clotic hydridist in particular, steed as the first produced and the charges are supported in the charge of the first type of incorporation for in, charge of the lipsomen can hydridist as a phending of the first type of management-charges considered conjugates. Similarly, since each of the first type of management-charges considered and produces and the similar of the charge stands to the first type of management-charges considered as engagement and hydridist to a phending of the same of an another charge character does not be formed. Also, the lipsoment surple behaviored to the same produces and to be detected. The implification provided by the samilarly behavioration samy under the charge detectable of the first sense or may increase the magnitude of the detectable change. This supplification is serviced in the assembly of medical and of the detectable change. This supplification is serviced in the assembly of medical and of the detectable change. This supplification is serviced in the assembly of medical and of the assembly of medical and of the detectable change. This supplification is serviced in the assembly of medical and of the detectable change. This supplification is serviced in the assembly of medical and of the detectable change. This supplification is serviced in the service of medical and of medical and of the service of the service of medical and of medical and of the service of the service of medical and of medical and of the service of the service

If desired, additional layers of nasoparticles can be built up by successive 25 additional crite file and second types of ansoparticle oligonacteristic conjugates. In distruse, the number of naroparticles immobilized per molecules of target number and can be further increased: with a corresponding increase in the innexity of the signal. Also, instead of using second and third type of comparaticle oligonacticotics.

conjugates designed to hybridize to each other directly, reconstrictes bearing
obigonucles) des that would serve to being the nanoparticles regether as a consequence
of hybridization with birding uligonucleotides could be used.

PCT/US00/01199

Methods of making the memograticies and the disputacionistics and of restricting the objectment of the memograticies and the disputacionistic metal restriction of adjustment of the memogratic method and which are disputationistic metal restriction and the state of the state of

An anample of this method of detecting satisfies and is illumented in Figure 7. The hydricitions of off facts up of or fine facts upon of a magnitude objectment of configuration to lippostories may produce a detectable change. In the case of gold nanopacticities, a plainful color may be observed for an pumple follow color may be observed for some complete infects or the preferration of the decorated for extraordinate objects on except in pumple. The hydricition is of the decorate type of management-of-digmentation decopyrates will produce an factoristic decign, making color pages with produce and celestrated facings. In the case of gold

oligonucloritie conjugates will produce a écistable charge. In the case of gold autoparticles, a purple/blue color will be observed. All of these color changes may be observed with the naked eye.

In yet other embodiments utilizing a arbstante, an "aggregate probe" can be

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on each of its two type of managemists has negativen by which is complementary to the segment of the primited of the molecular of the section. If the second type of alignment-involves on the first type of assignment-involves on which has necessary or which has necessary or a which has complementary to the sequence of of the second prior of alignment-involves on the 5 second type of assignment-involves on the primited by the first prior of alignment-involves on the sequence of the second of the second

containing numerous nanoparticles of both types. The aggregate probe can be utilized to detect nucleic acid in any of the above assay formats performed on a substrate, eliminating the need to build up layers of individual nanoparticles in order to obtain or enhance a detectable change. To over further enhance the detectable change, layers of aggregate probes can be built up by using two types of aggregate probes, the first type of aggregate probe having 15 objects elected a stacked to it that are complementary to objects of the other type of aggregate probe. In particular, when the aggregate probe is propared as illustrated in Pigure 28B, the aggregate probes can hybridize to each other to form the multiple layers. Some of the possible assay formats utilizing aggregate probos sec illustrated in Figures 28C-D. For instance, a type of oligonacteorides comprising 20 sequence a is attached to a substrate (see Figure 2BC). Sequence a is complementary to the sequence of of a portion of a muchic sold to be detected. The target nucleic acid is added and allowed to hybridize to the oligonucleorides attached to the substrate, after which the aggregate probe is added and allowed to hybridize to the portion of the target nucleic soid having requence h"; thereby producing a detectable 25 change. Alternatively, the target muelcic acid can first be hybridized to the approprie probe in solution and subsequently hybridized to the oligonacleotides on the substrate, or the target nucleic acid can simultaneously be hybridized to the aggregate probe and the offiguracleotides on the substrate. In another embediment, the target modele acid is allowed to react with the aggregate probe and another type of nanoparticles in 30 solution (see Figure 28D). Sume of the oligonucleatides attached to this additional type of praggaticles comprise sequence e so that they hybridize to sequence e' of the

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target modeiu axid and some of the oligonusteoxides statched to this additional type of emmognities comprise sequence d so that they can subsequently hybridize to uligonuscleoxides comprising sequence of which are attached to the substants. The core isself can also be used or a people to detect motivie scide. One

- 5 possible sassy formed is illustrated in Figure 2RE. An Electrated there, a type of oligomacheolides comprising sequence is a statished to a substrate. Sequence is it complementarily to the sequence is of a portion of a marker and to be detected. The target materials with a contacted with the substrates and allowed to hybridize to the originarization attained on the advancest. Then, another type of transparticies.
- 10 added. Some of the oil genusteesides attended to this additional type of numoparticles comprise sequence c so which is complementary to sequence of oil for turney under easily so that the numaparticle to philide so the surgest number is called bown the me substate. Some of the objectuate coldes with the deficient to the additional type of numoparticle substances and "on other numoparticles comprise supervisers and "on other numoparticles to sequence and and "on other numoparticles comprises supervisers and "on other numbers."
- 15 once prothe, and the core probe is added and allowed be hybridize to the eligenticisolides on the annoparticists. Since each core probe has responsers a and o' attends to the suspaparticists which comprise the core, the core probes can hybridize to each other to form unskiple layers attended to the activator, providing a greatly columned detectable closury. In advantage endorfermine, the target motives
- 20 could be contacted with the additional type of reimportaines in solution poles to bring consected with the substrate, or the target motion acid, the mospatificiat and the substrate control all be contacted insulmencesty. In you exastive affecting the additional type of reimportation could be replaced by a linking oligonate-order comprising both sequences and a few 2.
- 25 When a substrate is temployed, a plurality of the initial types of numerounicle-eligenatic oxide conjugate or eligenacionides can be attached to the substantes on any Pic detecting multiple portions of a target nucleis each, for detecting multiple decidence to the substantes of different nucleis day, or both. Design provides with rown of aprox, each aport containing a different type of oligenationide or of agents health.
- 30 nanoparticle conjugate designed to bind to a portion of a target audicie acid. A sample containing one or more nucleic acids is applied to each spot, and the rest of

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# WO 01/05/1665

PCT/US01/91190

the easiny is performed in one of the ways described above using appropriate all genucleotide-nanoponicle conjugates, oli genucleotide-liponome conjugates, aggregate probes, core probes, and binding oli genucleotides.

- Finally, when a substant is employed, it detectable change can be produced by Substant in employed with any produced produced by the standing. Silver staining can be employed with any produced that catalyze the reduction of allvir. Preferred are assopantiales under of mobile ments (e.g., gold and silver). See Bassell, et al., J. Cell Biol., 128, 80-876 (1990), Element-Production of all production production of a silvering see, 13, 228-251 (1992). If the
- manaparticles being employed for the detection of a motivic sold do not catalyze the 10 reduction of aliver, the nilver ions can be complexed to the runckie sold to catalyze the reduction. See Element at al., Natures, 301, 775 (1983). Also, Liver stains are known which can reset with the phosphate groups on uncertic solds.

Silver staining can be used to produce or obsence a describble change in any axes pre-present on a settentic, six-being slowed describbed shows: a particular, silver 15 saining has been found to provide a large processe in sensitivity for seasys enableping a single type of neospaticies, such as the one liberated in Figure 25A, so that the use of Bayers of sneopatricles, suggested probes and one probes can onless deliminated. In assets of determinated.

- change on he observed with an optical senses. Soliable senses include these used:

  20 to some documents into economic which are equisite of opporting in the reflective
  used (e.g., a flat document, observed one quick or greatering bit flattering to the control of the same type of optics, any type of greyeacle-centritive measurement,
  device, and standard senses which have been modified to involve to making the measurement
  to the inverteding c.g., a flatted senses modified to involve to table for the naturation.
- 25 to death, I have not bose found possible in war extraorer operating in the insummission made). The resolution on the possible resurt to custificate to that the exaction sees on the substrates is larger than a single pixel of the sourcer. The commer can be used with any substrate, provided that the detectable things produced by the susy can be observed against its substrate (as, party oper, such that specificate) parliers.
- 30 staining, can be observed against a white background, but cannot be observed against a grey hackground). The science can be a black-and-white actioner or, preferably, a

PCT/US01/01196

color account. Most preferably, the scanner is a standard color scanner of the type used to scan documents into computers. Such scanners are inexpensive and readily available commercially. For instance, an Epson Expression 636 (600 x 600 dpi), a UMAX Astra 1200 (300 x 300 dpi), or a Microtec 1600 (1600 x 1600 dpi) can be 5 med. The scenaer is linked to a computer loaded with software for processing the imeges obtained by searning the substrate. The software can be standard software which is readily available commercially, such as Adobe Photoshop 5.2 and Corel Photopaint 8.0. Using the software to calculate greyocale measurements provides a means of quantitating the results of the assays. The software can also provide a color 10 number for colored spots and est, generate images (e.g., printouts) of the scans which can be reviewed to provide a qualitative determination of the presence of a mucleic seld, the quantity of a nucleic said, or both. In addition, it has been found that the sensitivity of sessays such as that described in Example 5 can be increased by subtracting the color text represents a negative result (red in Fixample 5) from the 15 color that represents a positive result (blue in Example 5). The computer can be a standard personal computer which is readily available commercially. Thus, the use of a steedard scanner linked to a standard computer loaded with standard software can provide a convenient, easy, inexpensive means of detecting and quantitating mucleic neids when the assays are performed on substrates. The scans can also be stored in 20 the computer to maintain a record of the results for further reference or use. Of course, more sophisticated instruments and software can be used, if desired. A nanoparticle-oligonocleotide conjugate which may be used in an assay for any nucleic sold is illustrated in Figures 17D-E. This "universal probe" has oligonucleatides of a single sequence setsched to it. These oligonucleatides can 25 hybridizo with a binding oligonucleotide which has a sequence comprising at least two portions. The first portion is complementary to at least a portion of the sequence of the oligonucleotides on the ranoparticles. The second poetion is complementary to a portion of the sequence of the modelo acid to be detected. A plurality of binding oligonictections having the same liest portion and different second portions can be 30 used, in which case the "universal probe", after hybridization to the binding

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oligonucleotides, can bind to multiple portions of the nucleic said to be detected or to different medic acid targets.

- La a minber of other endodimens of the invention, the describble charges is exceed by helding the objection of the composition, the vide with motionize of section of the composition of the vide motionized of the composition of the vide motionized of the produced entertainty in the composition will be under management of the entertainty in the composition of the composition
- In anosparatics, dissurability quantities of the innocessories, over input now. Lemma disposabilities that only wire for in large in flowesterons, it is bette until the flowerstant groups are moved for accept nearly 6 on the recognities excludes on a sincreast in land makes in an long understand. Useful regular of designational and produce the control of the contr

measuring flateroisenes are well known in the art. Spillable Discretization and cache are also well known in the art and include the flaterocation, inclamations and Texes Red.

The obligance-considers will be attached on the nanosparincies as described showed for the control of the control

particles (stuch as polystyrune particles, polyyvinyt particles, eczylate and methacrylate particles), §lises particles, tetes particles, Sephacree bends and others like particles well known in the srt. Methods of standards oligomethotides to nuch particles are well known in the srt. Sec Christry et al., Nucleic Acids Research, 24, 3001-3039

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(1996) (glass) and Charpers et al., Augument, 13, 102-110 (1977), Rhys et al., Monlec cited his descript, 14, 110-130 (1970), Ellisoni et al., & Colomb Impriger 63, 20, 213-140 (1976), Kalmers et al., Biocoliniquez, 20, 15-14 (1976) (1976), Kalmers et al., Biocoliniquez, 20, 15-14 (1976) and Worlf et al., Nuclean discherment, 15, 213-140 (1971) (polymerimetre, 1s al. particular, videa verious of inscripted groups are available to not periodise cean he incorporated inscripted periodics. Particular groups inclined arthropic better days although a sinkey groups, course, period, without proper, Storyer groups, estable in a sinkey groups, sinkey groups, and periodic arthropic descriptions and the last Auguments and the last National Auguments and the last Natio

nanoparticles, can also be yand. The two fluorophores are designated d and a for Conor and acceptor. A variety of fluorescent molecules useful in such combinations are well known in for art and are available from, e.g., Molecular Probes. An attractive combination is Brorescein as the denor and Texas Red as acceptor. The two types of manoparticleoligonacleotide conjugates with d and a attached are mixed with the target anticip 15 acid, and fluorescence measured in a fluorimeter. The mixture will be excited with light of the wavelength that excites d, and the maxime will be monitored for fluorescence from a. Upon hybridization, d and a will be brought in proximity (see Figure 20B). In the case of non-metallic, non-semiconductor particles, hybridization will be shown by a shift in Eucoescence from that for d to that for a or by the 20 appearance of fincrescence for a in addition to that for d. In the absence of hybridization, the fluophores will be too far apart for energy transfer to be significant, and only the fluorescence of d will be observed. In the case of metallic and semiconductor nanoparticles, lack of hybridization will be shown by a lack of fluorescence due to d or a bocause of quenching (see shove). Hybridio will be 25 shown by an increase in fluorescence due to a.

As will be appreciated, the above described particles and managements having obligantationides liability with acceptance and down if numerous met molecular statebal can be used in the asystement described above, including these perimental provisions and on arbitrates. For solution formats, the object-to-level expounded are preferrably obligates in the trip principle of the tripper management in Figure 13.4-4. In the format above met Figure 13.4-4 and it, the healing object-to-level exposure of the formation of the Figure 13.4-4 and it, the healing object-to-level exposure of the formation of the Figure 13.4-4 and it, the healing object-to-level exposure of the formation of the Figure 13.4-4 and it, the healing object-to-level exposure of the figure 13.4-4 and it.

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to being this acceptor and ofonce Dourseous's moleculars on the two amongenicides in preceding. Also, in the format liberated in Figure 13A, the oligoneechoolides strukture abbusture any be biolated with A. Pardure, other bosts besides thoseocous molecules can be used, such as demolutizationers molecules, which will give a 5 deschable legal or a change in declarable spirit or a change in declarable spirit or a change in declarable spirit or process.

- Another embodianost of the detection method of the invention is a very sensitive system that utilizes detection of chappe in fluorescence and color (filastizated in Figure 21). This system employs fasts, microspheres to which are attached oliganuclocities labeted with a fluorescent molecule and gold senopartic is:
- 10 to which are state-bit oligouscleotides. The oligouscleotide sunrequeriele copingstes can be prepared as observibles above. Methods of state-bits oligouscleotides to heave adiceophrees are well known (see, e.g., Cherrype et al., Lengmunt, 13.3103-3110 (1977); Elissari et al., J. Colloid Burgiere Sci., 200.251 (200 (1987)), as see methods of labeling oligouscleotides with fluorescent soute-size (see above). The
- 30 offspecification as the factors interruptions on the decignocalization can the gold immograticity has response may able of high call for sequence of a traget market can be gold interruption. On the case of the department of a traget market can be reported as the designation of the case o
- ant quench many florotylors molecules since gold recognificate how very large polygonies coefficients. Thus, the florotestense of a solution contining metalic and 25 will be provided to the continuity of the continuity, with a reduction, me, estimatestion of, florotestense indicating a positive result. Profestivity, florower, the results of the starsy are detected by plusing a diopit of the solution eates a microprotest started (see Figure 23). The microprocess material should be
- transparent or a color  $(e_{i}K_{i}, white)$  which allows for detection of the pinkhed color of 30 the gold newoperfields. The microporous material should also have a pore size sufficiently large to allow the gold management to pass through the poten and

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antificiently small to retain the latex microspheres on the surface of the microporous material when the micropotous material is washed. Thus, when using such a microporous material, the size (dismeter) of the latex microspheres must be larger than the wize (diameter) of the gold nanoparticles. The microporous material must 5 also be inert to biological media. Many suitable microporous materials are known in the art and include various filters and membranes, such as modified polyvinylidens fluoride (PVDF, such as Durapore membrane filters purchased from Millipore Corp.) and pure cellulose scente (such as AcetatePlus "M membrase filters purchased from Micron Separations Inc.). Such a microporous maternal retains the actwork 10 composed of target mutitie acid and the two probes, and a positive result (presence of the target nucleic acid) is evidenced by a redipink color (due to the presence of the gold manoparticles) and a luck of fluorescence (due to quanching of fluorescence by the sold nancoarticles) (see Figure 21). A negative result (so target nucleic acid present) is evidenced by a white color and fluorescence, because the gold 15 aeroparticles would pass through the pores of the microporous material when it is washed (so no quenching of the fluorescence would occur), and the white letex microspheres would be trapped on top of it (see Figure 21). In addition, in the case of a positive result, changes in fluorescence and color can be observed as a function of temperature. For instance, as the temperature is raised, florerscence will be charred once the dehybridization temperature has been reached. Therefore, by looking at color or fluurescence as a function of temperature, information can be obtained about the degree of complementarity between the origonucleotide probes and the unget medeic acid. As noted above, this detection method exhibits high sensitivity. As little as 3 ferntemoles of single-stranded target nucleic acid 24 bases in length and 20 25 femtomores of double-stranded target sucleic acid 24 bases in length have been detected with the naked eye. The method is also very sample to use. Fluores

eye. Alternitively, for a more quantitative result, a fluorimeter cut be employed in 30 front-face mode to missure the fluorescence of the solution with a short pathlength.

can be generated by simply illuminating the solution or microporous material with a UV Imm, and the fluorescent and coloristentic signals can be monitored by the naked

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The above unbedients hat been described with particular reference to him incompletes and poli transporticles. A price interception of price in the color properties force hed drive and to which of ligarout-contracts can be attended.

5 described price of these particles. Meany untrivide particles and composition for the addition, microspheres and assumption having other reception group tens and yet used. For internet, projection may be used. The internet, projection may be used. The internet, projection end to the contract of the

In yet another embodiment, two probes comprising metallic or semiconductor 15 nanoparticles having oligonucleotides labeled with fluorescent molecules attached to thom are employed (illustrated in Pigure 22). The oligorus leotide-nanoparticle conjugates can be prepared and labeled with fluorescent molecules as described above. The olisumschoolides on the two types of oligonucleotico-nanoparticle conjugates have sequences espaids of hybridizing with different portions of the 20 requence of a target mudeic acid, but not with each other. When a target nucleic acid comprising sequences complementary to the sequences of the oligonucleotides on the nanoparticles is contented with the two probes, a network structure is formed (see Figure 22). Due to the quenching properties of the metallic or semiconductor nanoparticles, the fluorescence of the oligonucleotides attached to the nanoparticles in 25 quenched while part of this network. Thus, the fluorescence of a solution containing nucleic acid and the two probes can be monitored to detect the results, with a reduction in, or elimination of, flaurescence indicating a positive result. Preferably, however, the results of the assay are detected by placing a droplet of the solution onto a microporous material (see Figure 22). The microporous material should have a pos-30 alze sufficiently large to allow the amopurticles to pass through the pores and sufficiently small to retain the network on the surface of the microporous material

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when the new-process natural is switched (see Figure 12). Many missible miscoporous materials has where for new process of the miscoporous materials have been from the end thought how develoted above. Such a miscoporous material rations the network computed for larger structions shall not be many purples, and a positive count (presents of the larger structions) for before county from the processor (plus to spendholey of discoverances by the materials or misconducture supportation) (see Figure 22). A regular termit for usual processor (plus to spendholey 22). A regular termit for usual processor developed in the processor of the misconducture structure. A regular termit for the fluorest control of the fluorest terminal through the larger terminal to the larger terminal to the larger terminal to the larger terminal terminal to the larger terminal terminal terminal to the larger terminal termina

once the delight-filluration temperature has been transition. Thesetoric by Downing 41 to Minescreence as a Tentricum of Incompeting, and Communication can be obtained obtained the 5st degree of Complicementally however the delignoselocotic protess and that traps and cold. Processescence can be pareassed by prifily Hissinalizing the action of microprocero waterful with a 1VV lamp, and the Recovered signed can be removined by the mixed op 5. All mixed of 50. All mixed on 50. All mixed on 50. All mixed of 50. All

In yet other enhodiments, a "washilite probe" is used (see Figure 34). The smellite probe comprises a central particle with one or several phytical geographic tust can be explosed fin describe in an assay for muchic smile (e.g., intense other, subsectencing controlling skilly, majoration, Suitable particles include the 25 amongravitus and other particles destribuil above. The particle has wilgomedentical 26 amongravitus and other particles destribuil above. The particle has wilgomedentical 27 amongravitus and other particles destribuil above.

(all having the same required) amethed to it (see Figure 24). Morboda of strucking objective to the particle are described above. There objects reduced concepts as the safe payments and seemed portise, but of which the zero complementary we produce of the expenses of a surge scubic seed (see Figure 24). The weithing whose abso comprises good or disputational force Figure 24). The weithing whose abso comprises good originary actional facility and of the suppose of a surge scenario (see Figure 24). The objects are a first first grade and surgestion facilities are first grade and surgestion and a second portion (see Figure 24). The

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sequence of the first portion of the positive oligonocitedities in complementary to the first portion of the sequence of the eliquocitedities in complementary to the first portion of the sequence is municipated on the certain particle (see Figure 20.). Consequently, when the central particle sed the proble eliquocities are homely in an oceanor. The alignomicatedities are the particle of legislation portion in the position of the proble oligonocitedities are the particle of legislation of the proble oligonocitedities are complementary to profition of the requence of the traps antion of the Figure 20.). Each proble oligonocitedities is blacked with a reporter molecule (see Figure 22.). Each proble oligonocitedities is blacked with a reporter molecule (see Figure 22.). As selected described (see Figure 23.). See proble oligonocities is blacked with a reporter molecule (see Figure 22.) as forter described (see Figure 23.).

10 oligonucleoides and the ineged (Neugh of the portion hybridinod) is as large as, or genere that, the hybridinos overlap between the probe oligonucleoides and the oligonucleoides much be to the particle (see Figure 20). Therefore, temperature cycling resulting in dehybridization and rehybridization would flow moving the probe oligonucleoides from the centaril particle to the target. Then, the particles are speciated from one pole oligonucleoides when the centary that the reports are presented from one pole oligonucleoides hybridized to the ranget, and the response

15 separated from the probe oligonacleoides hybridized to the target, and the reporter molecule is detected.
The natellite probe can be used in a variety of detection strategies. For example, if the control perticle less a reagnetic core: and is covered with a material

capable of quonohing, the flowerscenes of floorophores attached to the probe of millipromotionides the automath, this system can be used in an in aira floorometric detection administration produced in the control of the control of

25 (1998))zsing will-d-erelapsel rifam surfice o femilistry (Chrisery et al., Abediel. Action Research, 24, 3031-3059 (1996)) and employed as magnetic probert as well. Festime, the dys motions, 44(-4(d-interhinalize)pharty); acceptomation and GOASCVL) has been shown to be an efficient operator of Directories for a wider variety of funcyclams started to objectoriested (Type) et al., Morne Biotech, 16, 49-53

30 (1998). The commercially-wallable succinimidal steer of DAECYL (Molecular
Probes) from: extremely stable unite bonds upon reaction with primary alkylamino

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group. Thus, no yeaponic provide or polymero-count anguaries provide with primary part of with middle with the objective count of the primary part of the middle of which and objective count of the three questions and the descript on the matter board objective count of the part of t

The comparation of the control of th

This opposes has one has further extended to an electrocommission strong variety and injunctioned assumpting passing consulpared to engagine state of the wides problem to engage state of a read-consulpared pared pared into engage state of a read-consulpared pared pared into engage state of a read-consulpared to engage state of a read-

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bromshexylferrocene is stirred in an aqueous FMPA solution at 120°C for 6 hours to from 6-hydroxyltasylferrocene is an added to a THF solution of NN-distropreyletsylsonise and bette cyanochyl+NN-distropreypletsylsonise and bette cyanochyl+NN-distropreypletsylsonise and bette cyanochyl+NN-distropreypletsylsonise and settle cyanochyl+NN-distropreypletsylsonise and settle specific properties of the forecast properties of the

- 5 Oligomententies motified polysors-coated gold manoprinists, where the polymer contains electro-chamically-active ferroener molecules, could ask to be unified. Watson et al., J. Am. Class, Soc. 1, 11, 462-463 (1995). A copolymer of amino reactive sites (e.g., enhydrides) could be incorporated size the polymer for reaction with muno-modified disposate toless. Most as 4, Bioconjugar Class. 6, 174-176 (1995) in
- 10 the presence of sayet and with temperature cycling, the rudow-active probe oligomecheolidus will move from the statilitie probe to the target. Came this has heppende, application of the unagentic field will remove the magnatic particle from mobilion leaving behind the redone-active probe oligomecheolidus hybridized with the target meletie relail. The amount of target thin can be determined by cyclic
- 15 woltanmetry or any electrochemical technique that can interrogate the redox-active molecule.

In yet another embeddenest of the Investion, a mobile axid as detected by contacting the nucleo add with a substrate. In-in-in-piciporal confider attached showers. The oligonateleotides have a sequence complementary to a first portion of the segment of the socialis and. The oligonateleotides are beated between a pair of

- 20 responses of the suchies man. The neighbourdedness are benefits or recovered to the control of the substant. The neighbourdedness are benefits or recovered to include on the substant or the matter of an anterioral mobile in social a constant or of describedly (e.g., glass, quarta, polymore, planties). The effectivedes may be made of any sunstand material (e.g., ments), such as gold, plantium, in suddo). The electrorides can be followingthed by conventionant microridednessing the following supplied (i.e., Thompson et al., eds. techniques).
- 25 techniques Sea, e.g., Inconduction To Microdifungupule (L. F. Thompston et al., e.f. American Chronical Society, Wangampa, D.C. 1983). The substrate away have a plansity of pairs of electrodes baused on it in an entry to allow for the detection of multiple portions of a single maleia each, the describes of multiple different meteliciancies, or both. Arrays of electrodes can be purchased (e.g., from Adhestedisciantific, acide, or describes of multiple different meteliciantific.)
- 30 Inc., Richmont, Virginia) or can be made by conventional microfibrication techniques. See, e.g., Introduction To Microlithography (LF, Thompson et al., eds.,

#### IN T/J SALAH 190

Asserteur Chemical Society, Washington, D.C. 1983). Suitable photomorks for ranking the serrys can be purchased (e.g., from Photomics, Whijting, GA). Each of the pairs of olectrodes in the surrey will have a type of oligonoclothides anached to the substrate between the two electrodes. The contacting takes place under conditions

- 5 effective to allow hybridization of the alignmenhorides on the substant with the metrics evid. Thus, the suchies and bound to the inhatuse, is constant of with a type of nanosparticles. The comparaticles must be made of a material which can occubed electricity. Such nanoparticles must be made of smaller it, such as gold annoparticles materials. The manaparticles will have one or store.
- 10 Togeth of collegorationistics methods to time, at least one of the hypes of originazionistics having a suppress complemently to a scenario portion of this sequence of the truckies shell. The consensing these priors under conditions effective to size by hydrication of the dispusementation on the management and the market sent. If the market sent, in the market sent, in the market sent, in the market sent, in the market sent, and a house in the fauntiance of the assumption to the character sent sent sentences, and a charge in tendence of the sentence of the character sentence and sentences, and the sentences of the sentence of th
- 20 have been brund to the solvaints between the discribeding. For instance, when you'd managearticists are used, the minimize case by consecuted with fill where the described amongs his clean of the control where the described is not clean to the control where the described is not considered. Another way to do do not describ a title case where the deficient of extraple type of amongstructure is not utilificient, in some cases the first year of amongstructure is not utilificient, in some cases the difficient point of amongstructure is not utilificient, in some cases the first year of amongstructure to what the substructure is not utilificient, in some cases the difficient sound on the substructure with a second type of recognitical towards when the substructure have not provide complexation of the described in the substructure of the control of the described in the control of the control of the described in the substructure of the control of the described in the substructure of the described in the control of the described in the substructure of the substructu
- terming oligorateleoptics stateshed to them what have a sequence complementary to the oligonucleoptice on the first type of nanoparticies. The contacting will take place under conditions effective no that the oligonucleoptides on the second type of nanoparticle hybridize to those on the first type of oligonucleotides. If needed, or nanoparticle hybridize to those on the first type of oligonucleotides. If needed, or and the second of the seco
- 30 desired, additional layers of nanoparticles can be built up by alternately adding the first and second types of nanoparticles until a sufficient number of nanoparticles are

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associated to the substrate to cluse the circuit. Another alternative to building up individual layers of nanoparticles would be the use of an aggregate probe (see above).

The invention also provides kits for determing nucleic acids. In one embodiment, the ich comprises ar least one container, the container holding at least

- 5 we types of amosperatine having objector/certides anothed threno. The objector/certides in the first type of amosperatine have a sequence complementary to the sequence of a first portion of a nutrier acid. The objector/certides we the second type of nanoparticles have a sequence complementary to the expertee of a second portion of the second resolution and the second second portion of the second resolution acid.
- 10 oligomaticolidos having a sequence complementary to a third portion of the auction acid, the third portion being located between the first and second portions. The filter oligomaticotide may siste be provided in a separate container.

It is second embodiment, the his comprises a least two containes. The first containes below quantities being displaced interested an embed of containe below and the container below quantities being displaced interested and embodiment below and the second container below upsymites belowing applicated data standed deteres which have a suppress complementary to the septement of a second protein of the model of the below of the protein of the model of the below of the second of

ha mother alternative embodiment, the hits cun have the disponselections and anapopurities in represe combiners, and the objects clother would have so be stacked in the manaportistics prior to performing an array to detect a muchica usid. The objectmentation under the emergentials may be forticinalized so that the

25 oligonaciosidas can be attached to the nanoparticles. Alternatively, the oligonaciosidas anties nanoparticles may be provided in the kit without functional groups, in which case they must be functionalized prior to perfacining the annay.

In another amboditmon, the kit comprises at least one container. The

consider holds metallic or semiconductor panoparticles having oligonacleosides

30 attached thereto. The oligonacleosides have a sequence complementary to a portion

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of a nucleic acid and have fluorescent molecules attached to the ends of the oligometrolides not attached to the nanoparticles.

b. you confirm embodiement, the last comprises a workers, the confirment handles graduated factors comproprised the Temporary and the Comprises a section of the Comprises and the Comprises a section of the Comprises a section of the Comprises and the Comprise and the Comprises and the Comprise and the Comprise and the Comprises and the Comprise and the Comprises and the Comprise and the Comprise

sean of portion of the binding dispranchesolitic.

In another mechanisms, the time comprises as substants having alligenselectificities attacked disense which have a sequence complicationaries by the supports of a first papelos of a models and if. The bits able in inclusive a first constant or hability amountained below which have a sequence complication much be inclusive as face contained to the sequence of a second profile on of the models exist. The olloparcelections may have better one of different supportice, better and for disputated order as sequence complimentary to a person of the models (e.d., The his three in buffers a second containor hability amountained to the support of the models (e.d., The his three in buffers a second containor hability amountained to the support of the support the lawing of compositions stanted and work which have a vector of the originary-footfee stateded to the manageration in the first terminater.

In yet another embodiment, the kite can have the substant, oligomethodides, and manaparticles in septemts containers. The substant, oligomethodides, and manaparticles until never the opportunity attached to cash other potent amount of the potential of the opportunity as except to detect a method and. The enhance, oligomethodides need for the manaparticles may be functionalized see expedite the standards. Advantage of the manaparticles may be functionalized as expedited in standardses. Advantaged to

WO 01/051665

nanoparticles.

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the substrute, obgonizationides and/or nanopurticles may be provided in the kit without functional groups, in which case they must be functionalized prior to performing the

- In a further embodiment, the kit comprises a withstrate having originary broad is a stand director which have a sequence complementary to the supersect of a first period on a national limit. The kit has included a first conjument habing Separates beaving offigurus-localises attached thereton which have a supersect complementary to the sequence of a second portion of the smallest said and a socond constant habing a manageratical having at least a first type of ollipsomolected estatched illeretis, the first
- 10 ppc of special production forcing a charactery jerup statuted in the ead and seatherd to be a subgradient to be first enough each cause that the local production is serveriors. The kin ency extracts on that or examples on their conspices of their constants building a second type of anteroperation. In this may write comprise of their constants building a second type of anteroperation building or improvement of the second determined to be second type of 15 migrature confidence and the second second type of originate stated as the first type of unsoparation buring a sequence of a second type of complementary in the acqueries of the confidence in the rings of unsoparation buring a separation of complementary in the acqueries of the confidence in the rings of unsoparation buring a sequence of complementary in the acqueries of the confidence in the confidence of complementary in the acqueries of the confidence of the confidence
- In mother cohesistance, the late may comprise as National Northean Northean
- to it which have a organic complementary to o second portion of the sequence of the matrix self-end.

  In yet sutther embodiment, the kit may comprise a substate having object-motoride statuted to it. The objectment obtains have a sequence complementary

to the sequence of a first portion of a nucleic sold. The kit further includes a first \$66\$

WO 01/051645

PCT/LS01/01190

container holding an aggrugate probe. The aggregate probe comprises at least two types of ranoperticise lawing disparaciocides stateded to them. The mampuricises of the aggregate probe unbound to each other su needs of the hybridization of anese of the oligomoclovides attached to each of them. At least one of the types of

5 manaparticles of the aggregate probe has oligonucleotides attached thereto which have a sequence complementary to a second portion of the sequence of the medicic acid.

a suppose complementary to a second portion of the reporter of the method in fall. In an additional mendodament, the hist ways composite a substant being objectively as insulated to it and a first occuriate helding an appraise probe. The appropriate as team to exposite a first to a sense of the hybridization of none of the appropriate price are bound to setch devier in a sense of the hybridization of none of the appropriate price as bound to each of them. A totat on our first help report of the option of name of the appropriate price is an exposite of the hybridization of none of the appropriationary to a first profession of the appropriate first appropriate price of the supposite of the victor of an appropriate price of the number of the first type of objective decides statistical to them. The first type of objective decide statistical to them. The first type of objective decide statistical to them. The first type of objective decide statistical price in the first type of objective decide statistical price in the first type of objective decide that a sequence of the sequence of the improvious statistical confidence statistical price in the confidence of the sequence of the improvious statistical complications state when the confidence is an exposite to the confidence approach of the sequence of the improvious statistical complications and the second to the confidence and the second period of the sequence of the improvious statistical confidence and the second period of the sequence of the improvious statistical confidence and the second to t

to the solution?

20 In souther embediated, the like may comprise a retermine whech has
of-generalized text tracked to it. The off-generalized features are supermore complementary
in the recognises of lain presenter of assistant limit for its own supermore complementary
in the recognises of lain presenter of assistant limit for its own supermore strategy
container holding lipocomes having objectnicelesis strategy in the supermore of a second portion
of the architecture. The like finder subschool associated container and before present of the supermore of the su

30 to it which have a hydrophobic groups attached to the ends ant attached to the nanoparticles.

PCT/LS01/01190

In a father embodiment, the bit may comprise a five container building immogration between glorestanciellules and between The kits the forestens one or more subdidicant container, such container building is blander ollegementeration. Each publishing oligometeration has a five protein which has a require container building is blander ollegementeration. Each publishing oligometeration from a requirement of the sequence of oligometeration of the sequence of a portion of the sequence of a section protein of the building oligometerations and a second portion which has a sequence complementary to the sequence of a few section of the sequence of the sequence of the section of

complementary in the sequence of a perion of the motions stall.

In author sheeddoment, like my complete once or two continiers building two types of particles. The first type of pertileals twint an impossible standard therein which have a requirement complementary with a squency of the profine of a material case.

2004. The oligonomicalists are builded with an energy down on the ends not sent which the complete of the particles. The record proof of particles that an entry of the particles of the articles are considered to the particles. The record proof of particles with an entry complete on the continued at the particles. The oligonomicalists will be also continued to the particles. The oligonomicalists are builded with an energy complete on the continued at anticle state of the particles. The oranger denotes and acceptors may be fluorescent.

In a further embodiment, the bit comprises a fest container holding a type of lates zricouphens having objeouseleotides attracted therees. The objeouseleotides have sequence complementary to a first portion of the sequence of a succise soil and are labeled with a flowerseam selected. The bit also womprises a second container holding a type of gold manaparticles having oligoruschestides attached

PCT/LS01/01199

thereto. These oligorousloctides have a acqueeue complementary to a second portion of the security of the author acid

- In souther sub-distinct, the bit is comprises it face consister bothings film type of measilities commission between the consister installing and the consister installing commissions and consister installing commissions.

  5. The disputationable have a sequence complementary to first proteins of the sequence or all consister and not sub-table in furneerees materials. The list also comprise a security contained between the consistence of the consis
- pols. The studies period configuration and the studies as studies pols. The studies polse congenies and polse through studies being assisted better objection being acceptance of the studies of the stud
- In norther embediment, the list may comprise a consider holding are agreement probe. The agargase profe compress at time two types of managements that series objects objects and the agargase probe and conjugate included teached to them. The management is and a single probe are a mental of the objects as the contract of the objects as the contract of the objects as the contract of the objects objects of the objects objects of the objects
- In an additional embodiment, the kit may comprise a container halding an

  aggregate probe. The aggregate probe comprises at least two types of nanoparticles
  having oligonucleusiste attached to them. The nanoparticles of the aggregate probe

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are bound to stain other as a result of the hybridization of some of the oligomolecities attacked to such of them. At least use of the types of emoparticles of the aggregate probe has oligomortecities sitticient to it which have a hydrophobic group allathed to the out not attached to the nanoparticles.

5 by a notifur embodiment, the invention provides a kit composition as abstrate having located thorous at least one pair of decreased with a digaranteered as answhot to the abstrate between the electricists. In a predictived embodiment, the authorities at plantality of pairs of accordes attached to it in as entray to allow for the detections of monthly portions of a single montain each, the detection of artifulia portions of a single montain each, the detection of artifulia portion and as single montain each of the detection of artifulia portion and as single montain each of the detection of a single montain each of the detection of the

10 anded, or both.
 The ist many also combine other reagents and trens varieful for detecting turkele road. The reagents many include PCR reagents, reagents for inliver statisting, hydrodization reagent, buffers, and. One times which may be provided up and of the little includes a mid-in whose Open visualizing hydrodization spots as a TLC olles player. In microprocurs instantia, pringing a physical crustic, constainer, and demonstrate (for consoling hydridization and de-hydridization temperatures). Reagents for Interioralizing the microdization compared temperature play has be included in in the St.

The precipitation of aggregated recognition provides a recover of requesting a stream of recipitating a factor and recipitating and a factor and recipitation of the matter size and the factor and the recipitation of the matter size and the factor and the recipitation of the matter size and the factor and the recipitation of the matter size and the size a

The investion site provides a method of nanofabrication. The method occuprises providing at least one type of linking of gonorleodide having a selected

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sequence. A listing adjustmentation to and for instrubblichasis may have up obtained sequence and may a larged transition of the section. It has not provided sequence and may be indiced reader of solid residence of the section. The sequences described in solid features in the less, suggest at hydrother sections. The sequences described in solid residence for the labelity of sequences of the labelity of sequences of the section of the labelity of sequences of the section of the sect

The sequence of a linking oligonusebooide will have at least a first perfero and a second portion for binding to oligonucleotides on nanoparticles. The first, second or more binding portions of the linking oligonucleotide may have the same or different

1.5 Vall of this histing portions of a limiting eligence could have the same requirement, only a liquid type or admorpstite with olligisated bodies in the histing a comprimentary requirement and discrete need to resid to be read to form a maximum. It is not or ence the limited precision of a limiting eligence therefore the registers are discretely requirement to the read of the residence of a limiting eligence the residence of the limit of granulated have different sequences, then to use more management-oil-upscarded-residence of the limit of the residence of the limit o

The linking eligenterlevides and ranspaction-origonucleotide conjugates are
so contacted under conditions effective for hybridication of the oligonucleotides attribed
to the comparticles with the linking oligonucleotides to that a desired manumaterial or

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# WO 00/05/1665

#### PCT/LS06/01190

canostructure is formed wherein the manaparticles are held together by oligonaticestide connectors. These hybridisation conditions are well known in the set, and can be optimized for a particestic manafabrication scheme (see above). Stringers introductions conditions are preferred.

5 The investion also provide nonher and of a manifoliheration. This method comprises providing at least two types of antaparacited estignance bandle engingers.

The adjustmentations on the first type of antaparacited in the as a sequence complicancing to that of the adjustmentation is the second type of antaparacited in the adjustmentation of the first type of antaparacited. The complicancinary to that of the adjustmentation to the first type of antaparacited. The

autoparticle-oli genezionicie conjuguen are cuntantel under conditiona effective to allow bydeldeziene of line oligonostieniches en its autoperticles to sech olite en that is derbied uncentrali en commontreae in formed where this emportations are both segetter by oligonosioniste consenters. Again, these bybridization contilions are 15 well-known in the art and en to be optimized for a periodise morefelections scheme. In both amountablication aembood of the serviction, to use of management.

having one or score different types of oligonalization transland thereto is temperature. The number of different eligenactive idea mixical is a semapartiale and the lengths and sequences of the one or more oligonaric-rolldan will contribute to the rightly and structural features of the resulting amonasterials and seasotherstees. Also, to, but, shape and the headant congosition of the surreportation of the surreportation.

contribute to the proporties of the resulting manomaterials and autonotroverse. These properties include optical properties, epocleteronic proporties, electrochemical properties, establing in available solutions, porce and chemical size properties, indextonic properties, including in available solutions, porce and chemical size variation, ability to separate bisocrite unoleculos while variing us a filter, etc. The use contributes the properties of the proper

of auxitores of acceptations having different states, shapes another chemical compositions, as well as the use of nanoparalies having uniform sizes, shapes and chemical compositions, are contemplated. In either habituation method, the transporticles in the resulting nazomaterial or

30 uncontrusture are held together by eligonuclocide connectors. The sequences, lengths, and simulations of the oligonuclocide connectors, and the number of

WO 01/05/145

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different edipocariasis consessive present will contribute as the rightly and 
struckular general of the insummentation entransitives. If the ollogeneduritie 
connective is paticity double-manufact, into glidger can be increased by the use of a 
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An unasive cuttern for spacing corresponded to robots due addition of our force hairing configurations as illuments in Figure 2. The sequence of the hising all configurations of the hising and the second of the configuration of the configur

A further elaboration of the scheme für creating defined spaces between nanoparticles is illustrated in Figure 4. In this case a double strauded segment of 30 DNA or RNA containing overtunging ends is employed to the linking bigsperiodecide. Psycholarisation of the single-stranded, overhanging segments of the

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lisking oliganucleoride with the oligenucleorides attached to the nanoparticles affords multiple double-stranded oliganucleoride cross-links between the nanoparticles. Stiffer patentiaterials and nanostructures, or portions thereof, can be generated

- by melly rise griphe involved disputational connections to inview assequences. In Bening the first light made, on our years of these they primitiate present promises made [Denze, Pl. L. and Denze, Pl. P. Server, 338, 64-50 (1979) or the partice promises produce produce promises of an appearation by a promising riphe served consection by the promise produce produce produce produce mode of an appearation benefit or filterated the Figure 10. In 16 of express shown 5 Figure 10, one and of anosperities to evaluate the Paper 10 in 18 of express shown 5 Figure 10, one and of anosperities in conjugated with a demand commissing profition necessidated in oth other serv to conjugate with a complementary origeneoclosis containing partner medication. Anotherise of the objective containing partner medication. Anotherise of the objective plant produced to the produced partner containing partner medication.
- menced objectationed in Servation on hybridistries. These, the re-primitation of objectations of the Servation of the Servati
- (1992). Teamger, R.L. and Wu, T. J. Am Chaus. Soc., 117, 7323-7328 (1995).
  Prakash, G. and Kool, J. Am. Chem. Suc., 114, 3323-3327 (1993).
  For construction of narromaterials and suncostructures, it way to destrable in some cause for fool? the assembly in place by overlant consulations after formation of
- 25 the nanomatrial or anastrarems by hybrid states of the objects closic components. This can be accomplished by incorporating furtilizing groups that undergo a triggerul incremitalize rection into the objects closed. An example of a functional group for this purpose is a stift-moniformhounded group. This better demonstrated that me sufflemediates because does group and without hybridizard.
- 30 eligonuciosities reacity undergo cooss-linking on irradiation with ultraviolet light (340 nm) (Tawis, F.D. et al. (1995) J. Am. Chem. Soc. 117, 8785-8792).

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Afternatively, one could acceptly the ultiplecoment of a 5° O long (goop from an all plasmodolisty, and aft the 7 questions in associated by a requirement of the state of the

A related compling mensions to both the assembled management system in place utilized indiplemental relational behaviors, and incomplicated by a terminal follogical policy of companies and the companies and a foreign and a for

bronoaccy/sumino derivative by reaction with a Secondaryti acytiling agent.

A fourth coupling scheme to book the assemblies in place utilities oxidation of menoparticles buring oliganalconidas terminated by thiophosphory's groups. Mail additing agents, such as possissium tricted dar, postasium Errit-grande (see Gryzzzov and Laioingar, Archiefe Acids Research, 2, 11, 400) or 2003, no. experiente.

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In addition, the properties of the unnounatorials and nanostructures can be altered by incorporating into the interconnecting oligonucleotide chains organic and inorganic functions that are held in place by covalent attachment to the oligomedication chains. A wide variety of backbone, base and sugar modifications are 5 well known (see for example Uhlaneau, E., and Peyman, A. Chamical Reviews, 90, 544-584 (1990). Also, the oligonucleoside shains could be replaced by "Peptide Nucleic Acid" chains (PNA), in which the mucleoride bases are held by a polypeptice backbone (see Wittung, P. et al., Nature, 368, 561-563 (1994).

As can be seen from the foregoing, the nanofabrication method of the 10 invention is extremely versatile. By varying the length, sequence and stranderiness of the linking oligonuclockides, the number, length, and sequence of the binding portions of the linking oligonuclectides, the length, sequence and number of the oligonucleotides attached to the nanoparticles, the size, shape and chemical composition of the nanoparticles, the number and types of different linking. 15 oligonsoleotides and nanoparticles used, and the strandedness of the oligonsoleotide commencers, nanomaterials and nanosmuctures having a wide range of structures and properties can be prepared. These structures and properties can be varied further by cross-linking of the oligonucleotide connectors, by functionalizing the oligonucleotides, by backbone, base or sugar modifications of the oligonacteotides, or 20 by the use of peptide-nucleic scids.

The nanounterials and nanostructures that can be made by the nanofabrication method of the invention include nanoscale mechanical devices, separation membranes, bio-filters, and biocaips. It is contemptated that the resonancials and nanostructures of the invention can be used as chemical sensors, in computers, for 25 drug delivery, for protein engineering, and as templates for biosynthesis/namoutrocture

fabrication/directed assembly of other structures. See generally Seeman et al., New J. Chem., 17, 739 (1993) for other possible applications. The nanomaterials and asposituatures that can be made by the manofabrication method of the invention also can include electronic devices. Whether nucleic unids could transport electrons has

30 been the subject of substantial controversy. As shown in Example 21 below,

WO 01/05/1665

PCT/LS01/01190

asseparticles assembled by DNA conduct electricity (the DNA connectors function as semiconductors).

Finally, the invention provides exhibited of making imigat anappreticleoligomatoxitide coplegates. In the first such method, eligipscurfecturies were bound to 3 charged manaperitides to produce make manaparticle-disputationists compagness. Charged manaperitides include manaparticles made of metal, such as gold sunoparticles.

The method comprises providing eligentationals having conviously bound during a method providing a florensia and provident as those the encoprotises. It is moderlies and functional groups are those document florens for installing (i.e., by cleaning-tops or covinces) bordering objective-florens in consequenties. Parliamater, objective-florensia and provident in installing of a configurational florensia and absention. In installing of an experience of the significant constantly bound to that if or if such out to the time of the florensia of the coligenselectation as the contract of the consequence of the colinear objects of

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The facility energy is the sale solution must be sufficient to overcome as leave are unifor the describent equalston and facility entirely and the electronaries of the engineerly-changed engouncement for positively-changed engoyenestes of the regulatory-changed engoyenestes of the positively-changed engoyenestes, or the changes of the engineerly-changed engoyenestes of the engole-changed engole-changed

increased gradually over time, he have no found to give good results.

After steeding has still, the eligence-ficialized and nanoparations are included in
the net househor for an additional protect of times sufficient to allow are filled
additional sulplacementation to take the semantication of the term that the
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The collapsiane produced by our of the "rughe" one powh when flowed in he conditionally must be flashed as the season and the three "rughe" on the conditional and the flashed as the law flower power for the collapsiane and additive to the three reconstructions of the collapsiane flower on the curricus of the compensation which is always by the "rugh" and the collapsiane flower of the curricus of

room temperature and pH 7.0 gives good results.

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cus to determined comprisedly. Generally, a surface decisity of at least 10 piccanolodous<sup>11</sup> will be adequate to provide stable nanoparaticle oligomenteated conjugates. Perforably, the narkace desatty is at least 15 piccanolesteras<sup>2</sup>. Since the ability of the oligouschedules of the conjugates to hybridize with mostric socks and aligomelosteath express can be deminished if the native destiny is no great parts.

suchoe dessily is preferely no greater than about 35.40 picomoisiens<sup>2</sup>. As used herein, "suble" excars that, for up-oxide of a loss tix results after the conjugates or made, a majority of the oligomoisestates remain stacked to the manaparticles and the oligomoclosoides are able in hybridize with moclaic soid and

 oligonorbotide targets noder standard conditions encountered in methods of decesting musicionaria and methods of nanofabrication.

Antic how to the earlily, the nanopartice digenomicoles computes make typic in order and which over translately septem. See, e.g., Ensopress. See, e.g., Ensopress. S., e.g., El 1996 is the present application. In particular, due to the high princip cleaning of the conjugate, they o'll assends he to the segregates in the parameter of a regard material cell of originated could.—The temperature over which the aggregates from a dimension in the consecutionly beam faults of long tearners, and it integrates the important particul corresponding beam faults of long tearners, the directivity and assessivity of the enables of delection of the present invention. A might beam missacity of the enables of delection of the present invention. A might beam fault and to fill the present invention. A might beam formation of the control of delection of delec

casiquates. Although thate foruses were originally discovered in susuay performed in solution, the advantages of the use of those conjugates have been found to estude to sways performed on substrates, including donce in which only a single type of onlyings in used. 25 

Blue been found that the hybridization efficiency of manoparticles.

25 Ill has been your dat the hydrodrastics enrichery or insurprised obigousciderida which competite a new biscreamed designated allowed only of the use of recognition objectoridate which competite a recognition portion and a spacer portion. "Recognition objectoridate which competite a recognition objectoridate which competite a sequence objectoridate in the competition objectoridate in the recognition objectoridate in the recognition objectoridate in the recognition objectories and objectories objector

oligonucleotide target. In this embodiment, the recognition oligonacleotides competa recognition portion and a specier portion, and it is the recognition portion which

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hybridizes to the nucleic acid or oligonarhootide target. The spacer portion of the recognition oligonarhootids is designed so that it was found to the monquirticles. For instance, the spacer portion could have a mainly covaluatly bound to it, the underly complising a functional group which can hind to the tumoparticles. These are the

- 5 mem mineties and functional groups as described above. As a result of the binding of the spacer portion of the recognition originate indigenceleoside to the manuparticles, the recognition portion is speed every form the author of the interporticles and is more accessible for hybridization with its target. The length and acqueme of the spacer portion provisiting need species for the exceptions provising pood species for the exceptions provising most species of the exceptions.
- 10 ascorpations can be determined empirically. It has been found that a spacer portion comprising at least about 10 aschemides, proteinbly 10-30 numbershiets, gives good results. The spacer joint in may have any sequence which does not interfere with the which it is a subject of the recognition of illipanchesidates to become bound to the ammoprisides core a mobile of the recognition of the grant positions, the spacer portions about it may be a market of the comprehensions.
- 15 sequences complementary to each other, to that of the recognition alignmehoolides, or us that of the muelet-is said or obligamehoolide target of the recognition alignmehoolide. Preferrichly, the between ofth much under the speace profine are all adentics, all thyraines, all cytidiens, or all granitum, unten this would cause one of the problems just intentional. More prefer sity, the bases are all adentics or all dynamics. Now operferrishly the bases are all adentics or all dynamics.
  - It has furthe been found that the use of diluter oligonucleotides in addition to recognition oligonucleotides provides a mean of stiffcing the conjugates to give a desired level of hybridization. The diluters and recognition oligonucleotides have been found to statude to dis nanoparticles in about the atme proportion as their stall of in
- 23 the solution contexted with the management of the prepare the conjugates. Thus, the series of the distance in recognition agreement solution to the management of the control of the distance of the conjugates will participate in a desired number of hybridization events. The dilateral digenomicolation was just easy to superviewhich does not into firm with the shifty of the recognition of ignoraccionities to be bound to the management of the recognition of the processing of the proc
- 30 so bind to a meleic acid or oligomuzicotide target. For instance, the dilutest oligonulceotides should not have a sequence complementary to thet of the recognition

PC17LS01/01190

olignuchenties or to the or the metties end or olignuchenties to rapid of the secondation college-confection. The officient operationable are the imperation of a legislation better than the of the encognition olignuchentication on the interest of a legislation olignuchentication or the other college-time or olignuchentication or the other college-time of a college-confection feetings. If the 5 man, most periodicity, who on the same taking the interest produce of interest produces olignuchentication of the other college-time other college

As can be restilly appreciated, highly desirable nanoparticle-disponacheoide conjugate can be prepared by employing, all of the nationed described above. By doing so, stable conjugates with sidored hybridization abilitate can be produced. Any of the above conjugates can be, and are preferably, used in any of the

15 methods of detecting methods existed essentibed above, and the invention also provides a fet competing a contineer balding any of the above conjugates. In addition, the conjugates can be, and are preferably, used in any of the methods of manifelectation of the invention and the trathods of angustering motion solid.

It is to be noted that the term ""o": ""o" "rolly refers to one or susee of that

outly, Por countpols, "a characteristic reflect to one or more characteristic or of least
one characteristic. As such, the terms "o" (o" "o"). "One or more" and "a" base too!

we used inserchangeably bendis. As is also to be noted that the terms "enopplaing",
"studying", and "having" have been used terroringeable;
"studying", and "having" have been used terroringeable;

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# KXAMPLES

# Example 1: Preparation of Observed conside-Modified Gold Nanoparticles A. Preparation Of Gold Nanoparticles

5 Out of colored, 13 mm desired over present by reduction of EA/CL, with colored in Care of EA/CL, with Care of EA/CL, with colored in Care of EA/CL, with Care of

# B. Synthesis Of Oisonucleotides

20 Oligomolecticles were synthesized on a 1 micromole scale using a Milliagne. Expecte DNA reproductive an insight eclosure mode using phospharamidist eleminary. Ecleratice, Ec. (2) (Oligomoleculoide and Anadigues of Protection (1999) words (ML Product, Oxford, 1991). All polisitions were purchased from Milliagne (DNA synthesis grade). Arrange compling efficiency varied from \$1 or 93.9%, set the firest dimenburghistyl 20 (MAT) protections grow years and exceed from the collegence-to-circlet to a let a purificant.

For 3'-thinh-hilgonunbeotides, Thinh-Modifier C3 S-S CPG support was purebased from Gion Research and used in the automated synthesizer. During normal cleavage from the solid support (16 for at 55° C), 0.65 M dithir/directed (DTT) was added to the

WO 04/05/1665

PC171301/01190

NILOH solution to reduce the 3' disolfishe to the thiol. Before purification by reverse phase high pressure liquid chromstography (HPLC), excess DTT was removed by extraction with edgy accuse.

Text -5 and alignmentendes, 9-Thiol-Modifier Cyphosphoramidia reagent was 5 perhaps the control of the control

The top's processing group was not measured, which wild be profited toom.
Reverse place PIEC or was profession with a Distine DXXXI by system equipped with
a Newtieth Piccard OSIS hyperal robusts (64.5.100 mm, 5 mm) particle is sign winty OSI
AN THE ATT ON THE PICCARD AND IT AND IT Wishine a greated or SPAC EXCENSIVE
TEAN. The flow one save is and "with with 40 V describen as 250 mm. Preparative INELAwas and to particle DATT Provisions and marked a alignmentative (shows at 27 min.).
After collection and evaporation of the buffer, the DXTV varie affected one the
edignaturisheesis by reviewment with 80 V/M and a life or 90 min in versus requesters. The
28 enhances was those overpresent to the art dynam, writer was adult, and the cleaned DXTV
was extracted from the appearson of such 80 V/M and the SIA of 90 min in versus. The resonant
or of alignmentation was determined by alternative and 90 mm, and final purely sensested by
enterer packed PIECA (clination four 4.5 in such as 100 mm, and final purely sensested by
enterer packed PIECA (clination four 4.5 in such as 100 mm, and final purely sensested by
enterer packed PIECA (clination four 4.5 in such as 100 mm, and final purely sensested by
enterer packed PIECA (clination four 4.5 in such as 100 mm, and final purely sensested by

PCT/LS01/01190

The same protocol was used for purification of the X-third-of-geometrotides, except that DTT was added after extraction of DMT to reduce the amount of distrible founds. After its hours at 40°C, the DTT was extracted using ethyl acettas, and the obligamentoptides reputified by BPLC (elanion time 15 attaines).

For purification of the 5' thiol modified oligonucleotides, preparatory HPLC was performed under the same conditions as for sumedified oligonucleotides. After purification, the trityl protecting group was removed by adding 150 mL of a 50 mM AgNO<sub>3</sub> solution to the dry offgomulcotide sample. The sample named a milky white color as the elegyage occurred. After 20 minutes, 200 µL of a 10 mg/ml solution of DTT 10 was added to complex the Ag (five minute reaction time), and the sample was centrifuged to precipitate the yellow complex. The offgenucleotide solution (<50 OD) was then transferred onto a decalting NAP-5 culumn (Pharmacia Biotech, Uppsala, Swedon) for purification (contains DNA Grade Sephudes G-25 Medium for desalting and huffer exchange of oligonucleotides greater than 10 bases). The amount of 5' thiol modified 15 aligonucleoride was determined by UV-vis spectroscopy by measuring the magninule of the absorbance at 260 nm. The final purity was assessed by performing ion-exchange HPLC with a Dionex Nucleonan PA-100 (4 x 250) column using a 10 mM NaOH solution (pH 12) with a 2%/min gradient of 10 mM NaOH, 1M NaCl solution. Typically, two peaks resulted with elution times of approximately 19 manutes and 25 minutes 20 (clution times are dependent on the length of the offgorsedectide strand). These peaks corresponded to the third and the disulfide oligonucleotides respectively.

# C. Attachment Of Oligenucleoxides To Gold Nanoparticles

An augmous solution of 17th (150 µL) Au colloids, propered as described in part
A have, was nincel with 3.75 ph (46 µL) 3-fthje-17TAC/TCA, propused as described
to part B shall allowed boards for 24 hours at forme tompetate in an Im Rignordsort
aspect visit. A second solution of colloids was reserted with 3.75 µbd (46 µL) 3-thielTACCCTTCI. Note that there adigmane/backles are unaccomplementary. Sharlly before
these, social manages of each of the two neceptorized solutions were combined. Since the

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ofigoracleotides are noncomplementary, no reaction took place.

The oligonucleoside-modified macoparticles for smith at all-ersand temperatures (80°C) and high said concentrations (1M NaCI) for days and have not been observed to undergo particle growds. Stability in high said concentrations is important, since such 5 conditions are required for the hybridization rescribes that form the basis of the method of desention and sanotherization of the invention.

# Example 2: Formation Of Nanoparticle Aggregates

A. Proparation Of Linking Observatoride

10 Two (nonthibited) oligonucteotides were synthesized as described in part B of Example 1. They had the following sequences:

3' ATATGCGCGA TCTCAGCAAA (SEQ ID NO:1]; and 3' GATGGCGCAT ATCAACGGTA (SEQ ID NO:2).

Mining of these two oligocarthodore in a 1 M NGL, 10 mJs phosphate buffered 
(pld 7.0) solution, resulted in hybridistation to form a dupler haveing a 17-base-pair 
everlap and two 8-base-pair indict, rents. Leach of the existy-exists bad a requiremental 
was corruptomentary or that of one of the oligomoleosides attached to the Au colloids 
prepared in part of Example 1.

# B. Formation Of Nanoparticle Aggregates

The liability of general brokkes prepared in part A of this example (0.17 Jul film) connectoration that fillulation with NACIJ were added to the ranspart close-diagonal confidence consignates prepared to part C of Example (1.13 And Rant connectations there district with NoCI) at two management. The subsidies was then distinct with capacitas NACI (2 to a fill and connectation of 1 M) and buffered at 817 volvin (1 mad phorphata, conditions as which are cutable for bybridizations of the collegement confidence. An immediate color charge 52

which are usuable for hyporthization of the objective conjunction (i.e., an immensate coord crising); from red to pumple was observed, and a peccipitation reaction ensued. See Figure 6. Over the course of several hours, the solution become clear and a pinkish-gray peccipitate satisfied to the bottom of the reaction wereal. See Figure 6

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To verify that this process involved both the aliquim-consistes and solitols, the procipitus was collected and resupposed both shalling in 1 M acquest Del Inflered at pl 17. Any of the oliganus/societies not bybelisted to the nanoparticles are reserved in this answers. Then, a ser-personnel indusculation experiment was prediment by maintaining the characteristic characteristic production of the product ophicocory/societies (20 cm) und for the engagement culculati which is reflective of the productive distance (700 mm). See Figure 7.

Changes in shandarance at 200 and 500 to were recorded on a Patkin-Flator funded 2 UV-vis Spectosphotometer using a Pelder PPP-I reoperatuse Consolided Cell 10 Holder while spyling the temperature at a sate of T-Chanineb between UC and 40°C. DNA, solutions were approximately 1 absorbance until(s) (CD), buffered at pH 7 uning 10 and phosphotomic form and 1 MA Coll consentration.

The results are rivers in Figure 2A. As for Enumerative was option between O'C. and BPC (violed in 3PC closers the dissociation temperature (T<sub>2</sub>) for the outputs (T<sub>2</sub> = 15 "C)), there was an excellent constraint between the optical signatures for both the colloids and disjounce-footies. The UV-vis spectrum for naked Au colloids was much less temperature decondord. Figure 4.

Throw was a substainful visible optical change whom the polyment of agranosicodife-collected precipitate was based above 1st melting point. The clear tool mon 20 mand days for all so polyment is bounded in 6-byteridated to penerate the emittated collected which are adobted in the supercus solution. The process was reventible, as evidenced by the temperature traces in Figure SA.

In a control experiment, a 14-T:14-A dupter was shown to be indiffered to a limitating reversible Au willoid particle aggregation. In another control experiment, a 25 flasting origination dupter with the base pair assumated in the studyer on the set study on the set of the control of the set of t

WO 01/051665

duplexes.

PCT/LS01/01190

complementary to the citicky ends of the linking officerucleotide and reacted with nanoparticles did not produce reversible aggregation when the nanoparticles were combined with the linking oligoraclootide.

- Perhate evidence of the polymetation/steambly process come from Transmission Electron, Sciencepoy (TDB dosine of the precipities. TDM was performed on a Histolia 1010 Transmission Electron Microscope. A typical surpel was performed to a Histolia 1010 Transmission Electron Microscope. A typical surpel was personal by also polymetation on an a bring verbon godd. The grief, then, was dust under vocume and transget. TDM images of An cellulate fished by physicial collisions/science described networks of extend conflicts. In collision 102 also particular described from conflicts of excellular fished procedure of the procedure of procedure of procedure of procedure of procedure of procedure of the pro
- nemes to be remutably regular in view with an everage distance of 12 cms.

  With TRAL expression of layous desirbed, making in difficult surrish to design or of sele for intra-distanceaning aggregates. However, matter sells images of single layou, we confirmed an expression of layous process, Plazo 982. Choo-packed anounded on an ordinate of the end diseasement of the procession of the procession of the end of the procession of the end of the end of the procession of the end of th

Example 3: Preparation of Oligonacleotide-Modified Gold Nanoparticles

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Gold colloids (15 nm diameter) were propored as described in Example 1. Thiolologomecheolides [HS(CH:)+OP(CX(O')-oligomecheolides [HS(CH:)+OP(CX(O')-oligomecheolide)] were also prepared as described in Example 1.

- The method of attacking third-eligenucleoties to gold aneaparticle described S. Beampie. I was found not to produce satisfactory results in some cases. In particular, when he keep objugations were some of the approximation's collabor elegenature were not staked in the presence of a single series of high understain weight authors sports DNA and on arround the horizontail DNA has work ormally be present as fringing systems. Congret exposure of the collisides the intiel-eligenutheted for produced elegenature of the collision of the collision
- 10 oligonuscleotide-colleid cogingates that were stable to rathron aports DNA, but the resulting conjugates filled to hybridize selfactority. Further experimentation fed to the 610ming procedure for stanting third-oligonuclocides of may length to gold colloids so that the conjugates are stable to high molecular vertigin TNAI and hybridize stifulationally.
- A 1 This design of the gas be enabled (17 Mg) in verse run as much with eccess.

  O 48 30 Mg the Organization (27 Mg to Mg the Use of the Organization variety and the value of the values were allowed to a rand for (2.34 hours or room freegreeners. Then (10 Mg, 4 mg to 11 Mg the organization better, gain 2 Mg to 4 Mg to Mg to 12 Mg the Organization and added. After the organization, 19 Left of '18 species NNS; were added, and the selection, 19 Left of '18 species NNS; were added, and the selection, 19 Left of '18 species NNS; were added, and the selection of the organization of the organization to complete discovered the search of the organization of the org
  - nm), and a compact, dark, gelaticous residue at the bonom of the tabe. The supernatural was removed, and the recidue was resuspended in about 200 µL of buffer (10 mM

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phosphia, 0, 18 (NGC) and reconstrugate. After currous of the superments soldent, in tentiles we set interes in 10 Auf. of Marke (10 Aug. Aug. Aug.) and 10 Aug. of 15 15 a queezes soldierin of NNA). Dissolations was solicited by severing the soliton loss, and the expelliting it films, a player reversed into a. The entaling red mater solition was such as 5 (Aug., manifest end and did not suggraphic) on strateling red mater soliton loss seasons and the entality of the soliton of the entality of the entality

## 10 Frample 4: Acceleration Of Hybridization of Nanoporticle-Oligonacheolide Conjugates

The oligonuctivolids-gold collisid conjugator I and II illustrated in Figure II were prepared as described in Extraple 3. The hybridisation of those two codepates was extremely slow, in puricular, single grantee for conjugator I and III respectively. In puricular, single grantee for conjugator I and III respects 0.0.1 M NaCl and allowing the enixture to stend at room

temporation for a far yer/bond filler are notice change.

The way see fine the improve high distance. Furst, future scalar were observed by freezing the minimum of conjugates I send Leads to the deceasand as a some contract of Li MircCl in a dy too conjugate I send Lead to the deceasand as the size of the conjugates are some temporation. The thread evolution and thank chairs When I jill of the authors we promise on a standard C-1917 C-18 indep the Deliter of When I jill of the solution was spread on a standard C-1917 C-18 indep the Deliter of Minimum of the Confusion of the Confusion of the Send of the Confusion of the Send of the Minimum of the Send of the

solution was not refruzen, the spot obtained on the C-18 TLC plate was pink.

A second way to obtain fester results is to warm the conjugates and target. For

PCT/LS05/01190

instance, na morber experimente, deligentetenirle gold citalical conjugiere and an obspannisabilità seri protective a la 10 Marci Cauliniano vera servante agglery 65°C and allowed to cool to trans temperature over a period of 20 minotan. On opolitique on a California delicación del deligio para las pore indicative el 25 minotanicano vera debiande. Il an 5 minora, inclusión del con decempatame servar se mon temperature del nestre más 10 Minora NICL position del dos produces a base code ministrative or "hybridización. Hybridización in more repár (in 30 Minora).

## Example 5: Assays Using Namparticle-Ollaponsclastide Conjugates

- The oligomoteroide gold colleid conjugates i and 3 libroranda in Figura 12A-F were propored an desemble in Binample 3, and the oligomoteroide target 3 libroranda in Figura 12A-way prepared a described in Europeia 2. Minamedia cal deficients insept 4, 5, 4, and 7 were purchased from the Northwestern University Biocethology Fieldby, Chicago, II. These pilipomoteroides were psythesized on a 40 monit cells and printfolio an enterna plassa C18 encoding (CVCC). Their justicy was discussionally by performing one
- accinege ITHLC.
  Solective hydrolization was arbitreed by beating rapidly and then cooling motify
  to the stringment interpretature. For example, hybridization was carried use in 100 pil. of 23.

  MACI; plus 3 mM MgCQ; containing 15 and of create disjunctionally-ordered conjugated
  and 2, and 3 armonomious frategrat designmented at 3, 4, 5, 6, 7°, hearing to 7°C, cooling
  - to the temperatures indicated in Table 1 below, and incritating the mixture on this temperature for 10 minutes. A 3 pL sample of rach reaction mixture was then aposted on a C-18 TLC silica plain. On drying (5 minutes), a strong blue color appeared if hyphridization, had takes place.

The results are presented in Table I below Pink spots signify a negative text (i.e., that the ranoparticles were not brought together by hybridization, and blue spots signify a positive test (i.e., that the mosparticles were brough into proximity due to hybridization involving hole of the oligopastheristic-collisis conjugates.

WO 01/05/665

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TABLE 1

Reactionis	Results (Color)				
	45°C	50°C	99°C	74°C	
1+2	Pink	Pink	Pink	Pink	
1+2+3 (malch)	(Stue	Blue	Blue	Bitte	
1+2+4 (helf complement mismatch)	Pink	Pitk	Pira	Pink	
1+2+6(-6 bp)	Blue	Pink	Pink	Pirk	
1 + 2 + 6 (1 bp m/smetch)	Blue	Blue	Pink	Pirk	
1+2+7 (2 bp mismatch)	Pink	Pirk	Pins	Pirk	

As can be seen in Table 1, hybridization at 50°C yave a bine spot only for the fully-matched target 3. Hybridization at 50°C yielded blue apots with both targets 3 and 6. Hybridization at 45°C gave bite spots with targets 5,5 and 6.

In a related series, a brigger constaining a single microstabl T constraint was found to signify a positive test at 55°C (blue color) and a sengarive test (via color) at 6°C which conjugated a 15°C. Their the same conditions, the fully-matted target (3) gave a positive test at both temperatures, showing that he hear can discriminate between a terget that is fully matched and one constituting a single microstated base.

Single-restRow were soldered using a different hybridization unclock. In particular, solderile hybridization was mixed by froating, familier during washing by froating, familier during writing problems of the samples problems of the samples of th

WO 00051665

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TABLE 2

Reacturis (probes) « terget			Results (color)		
	RT	35°C	40°C	SI'C	64.0
(1+2)+3	pyre	blue	pite	bue	ptrik
(1+2)	penk	pirk	pink	pirk	gánk
(1+2)+4	rink	pins	plnk.	pink	pesk
(1+2)+5	blue	bue	pirk	pink	pente
(1+2)+6	biue	but	blue	pink	pink
(1+2)+7	blue	přek	pirk "	genk	pirox

An important feature of these systems was that the color change stanciated with the conpertance change was very shorp, occurring over a temperature many of should TC. This indicates high cooperatively in the melting and association processes involving the collect conjugates and candidate one to easily discriminate between objugates and candidate containing a Sifty-matched sequence and a single basepair minmatch.

The Night degree of Encironation may be embraced to now features. The first is adjusted on the interpretation of the transposance of the transposa

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for this detection approach is the temperature range for the colorimetric exponse (<1 °C) observe on the C18 utilize plates. In priciciple, this three-outspream naneparticle based strategy will be more relocally of them may two-component electrics system based on a single-strand grobe hybridizing with target runtile said.

5. A matter desiliden constituting 1 mode of trayer? A very represent in 190 of of hybridinesis hatter (23 M NoCA, 10 mod phosphates, pt 77). One p of feels authoris consequences for 0 phasmost and sagast dispusationable. Seleki dilutions were performed by index, or allowed of the moure relation and dilution is to the desired conventions with hybridisations build. The hobs below the security chostenesis step 1 per dismiture of 10 probed and 2 with different resources of trayers. 3. Allow performing the hybridisation which produces the continuous, 3 plat deposes for these substitutes resources and CLUE plants to determine colors. In Table 3 below, pinkt apparets an agentine tray and with the designation 5 positions.

TABLE 3

Amount of Terget	Results		
1 picomole	bke (positive)		
200 fermonole	blue (positive)		
100 femioracie	blue (gostive)		
212 fermomole	blue (positive)		
10 femianole	purprish (ambiguous)		

This experiment indicates that 10 femountoles is the lower limit of detection for this particular system.

Example 6: Assay L'sing Nappurticle-Oliganacicoside Cocinentes

DNA modified anaposaticles were néarboal onto modified transpasses substrates
as abown in Figure 13n. This method involved the lithéing of DNA modified
amospaticles to anaporaticles that we attached to a glass substrate, using DNA

25 hybridization interactions.

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DNA was amerbed to the assoparticle modified surface by sosking the glass sides in 0.2 OO (1.7 pM) solution commising femily purified 3' thick objects to the side of the control of the solution of the solut

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PCT/US01/01190

(prepared as described in Example 3) that is complementary to the subprished or princ of the linking objectoristic stateches to the substrate. After 12 hours of conting, the substrate was removed and missed with the hybridization buffler. The substrate color back distributed to a pumple color and the UV-vis absorbance at 320 nm approximately doubled 5 (Figure 14-A).

To verify that the oligomocleotide medified gold naneparticles were attached to the nilgonucleotide/manoparticle medified surface through DNA hybridization interactions with the linking oligomocleotide, a melting curve was performed. For the melting experiment, the substrate was placed in a coverte contaming 1 mf. of

- 10 Info@distanter wrifer and the same apparents send in Example 2, port 8, was used. The absorbance signal due to the manaparentical (200 arm) was promitioned as the temporation of the substantion was increased at a rare of 0.5°C per minuse. The manaparential gained demansicality dropped when the temporation passed OFC. See Figure 140: A first derivative of the signal showed a membring temporation of GCV, which corresponds with
- 15 the temperature seen for the three DNA sequences hybridized in solution without nanoparticles. See Figure 14B.

## Example 7: Assays Using Nanoparticle-Oliganic legitide Conjugates

The detection system illustrated in Figures 15.4-G was designed so that the two
probes 1 and 2 oilign is stall-to-stall flashion onto a complementary rarger 4 (see Figures
15.4-G). This differs from the system described in Example 5 where the two probes align
conditionally on the tracest stand (see Figures 12A-F).

The dignomolective gold managerische copilgates 1 and 2 illustrated in Figures 15A G were prepared as destructed in Example 1, recept that the anoxymicities were collegorated by infliciation buffer G all Am CL [1 and phosphore, ptf 7]. The field managerische-dignomoleculer conjugate concentration was estimated to be 13 Am by measuring the revierable is clearly of the surface plasmore band at 222 an which gives rise to the red core of the enarporticed trayes illustrated in

WO \$1051665

PCT/LS01/01190

Figures ISA-G were purchased from the Northwestern University Biotechnology Facility, Evension, IL

When 150 µL of hybridization buffer containing 13 aM oligonucleotidenanoparticle conjugates 1 and 2 was mixed with 60 picomoles (6  $\mu L$ ) of target 4, the 5 solution color immediately changed from red to pumple. This color change occurs as a result of the formation of large oligonucleotide-linked polymeric networks of gold nanoparticles, which leads to a red shift in the surface plasmon resonance of the nenoparticles. When the solution was allowed to stand for over 2 hours, precipitation of large macroscopic aggregates was observed. A 'melting analysis' of the solution with the 10 suspended aggregates was performed. To perform the 'molting analysis', the solution was diluted to 1 ml with hybridization buffer, and the optical signature of the aggregates at 260 nm was recorded at one mirrore intervals as the temperature was increased from 25°C to 75°C, with a holding time of 1 minute/degree. Consistent with chanacterization of the aggregate as an oligonucleotide-nanoparticle polymer, a characteristic sheep transition 15 (full width at half maximum, FW<sub>102</sub> of the first derivative = 3.5°C) was observed with a "molting temperature"  $(T_m)$  of 53.5°C. This compares well with the  $T_m$  associated with the broader transition observed for oligonacteorides without nanoparticles ( $T_{cr} = 54^{\circ}C_{s}$  $FW_{M2} \sim -13.5$ °C). The 'melting analysis' of the oligonucleatide solution without nanoparticles was performed under similar conditions as the analysis with nanoparticles, 20 except that the temperature was increased from 10-89 °C. Also, the solution was 1.04 µM in each oligenuclostide component.

To set the selectivity of the system, for T<sub>i</sub>, for the suggests formed from the profits complement 6 of product is and 2 was compared with the T<sub>i</sub>, for to graspeste famued from target that contained on these assimations, electrical, or is invertised (Figures 25 15A,G). Significantly, all of the gold management-be-digeometrical segments that consistent lespectric trapes enchloided against, maternalsh elembrishisten when compared to the paggraptic formed from the perfect complement, at relabored by T<sub>i</sub>, when for the visitions acquirated one River 25A-O<sub>2</sub>. The solutions containing the

PCT/LS01/01190

inspection tragens could usually be distinguished from the solution containing the profess complement by their colors when placed as a verter that hold at \$2.5°C. This temperature is above the 7.0°C in suite exthes opposite colories, to only on section with the profess target exhibited a pumple color at this temperature. A 'melting analysis' was also 5 performed on the probe colories which constrained the ball occupiementary stages. Only a missist to texture in characteristic at 100 and 100 complementary stages. Only a missist to texture in characteristic at 100 and 100 complementary stages.

ministrictoreas in forturbrene et al 20 mm was observed.

Nat. 2, 2, 1, 2 (2) generalistal et and esh on disponiteation targets (17-pers 13A-C)
were nefect to a solution containing 50 gli, of each provide (1) Acy) in bybrideristies breitly
contained to a solution containing 50 gli, of each provide (1) Acy) in bybrideristies breitly
to the containing the solution of the solution of the containing the solution of the contained to a fact the compensation contained variety to load undersided at the temperature contained variety to load on the containing the solution of the containing the solution of the

open signify is positive sets.

Nationly, he conformed instantion that can be detended by the naturel cyc occurs

on we have have 10°C, thereby allowing one to stank) distinguish the prifect sings of the naturel of and 0, and exhibition (1) and no to the sinestine and the point in the target where the two dispunded of produces med (10°C are Table 40.) Normal the ecolorismic training in 1°C and the site of price in 1°C and 1°C are the site of the

The results are presented in Table 4 below. Pink spots signify a negative test, and blue

showed negative spois (pink) in the plate test at all temperatures (Table 4).

The observation that the one base insertion target 8 can be differentiated from the fully complementary target 4 is truly remarkable given the complete complementarity of

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the hereiter used with the hereiter punks reporters. The disabilitation of the aggregate formed then at any form expecting planes are used to be the order of the aggregate formed to the size of the aggregate to be due to the order they also produce on the latest after the punks of the latest and the latest after the punks and the latest after the punks and except the punks and the latest and t

techniques.

The merital indicates that any one base reliananch along the striper resend can be defended, along with any stranscens lobe the trapel strans. I proporticity, the trought stranscent reage over which such changes can be destined in a stransport, when the first the destination of the change and the stransport of the stransp

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Reactants (protes) + target	Russilis (colur)					
	RT	47.6°C	60 6°C	81,4°C	52.7°C	64.510
(1+2)	pink	) plets	pink	pink	plak	pink
(1+2)+3	gánk	plete	pink.	pink	plak	pink
(1+2)+4	blue	bixe	blue	èlue	blue	pink
(1+2)+5	blue	btvo	blue	pink	pink	prnk
(1+2)+5	blue	pink	pirk	nirk .	pink	pínk
(1+2)+7	blue	Hue	blue	blue	oink	presk
(1+2)+8	blue	blue	plak	ark	pink	pink

WO 03/05/1665

possible.

PCT/LS01/01190

Exemple E. Assas Likes Nanomicki-Olimenolatic Collectate
As at al experients were performed inversing hydrideason with Black dayles
ediporatelesian. Nanoparticle ediporateleside conjugant 1 and 2 illuscrated as Figure
164 was inchand with surgest of different lengths CR, 48 and 73 bases in Integral
complementary filled informationalists, an infantion of Fagure 164-Co-Ombavine, the
confidence were in described in Exemple 7. Also, the ediporatelesides and sunoparticle
ediporatelesides or experience of the Exemple 7. Also, the ediporatelesides and sunoparticle
ediporateleside recipients were prepared as often 16 Exemple 7.

As recent, the difference rescribes adulting to the most off effects or opical properties that by biddentiation due to the district explorate deptices opicially configurated for be poil to amorparishes. See Table's below. However, when there trainings were reported onto a CHITCE plane, to but color developed upon drying and contrasportance or UFC, regardless of the raise of the facility designationalities and the finance trainings were the position amorparishes. See Table 5 The probably occurs because the notific import entitives reaggregation of the byth-dired obligomaticides immorphisms objects and the finance trainings are received to the contrasport of the district of the districts of the districts of the districts of the districts between the gold composition of the contrasting of the ATD Seeds, and decliminate forestories is will

The color changes observed in this and other examples occur when the distance between the gold pureparticles (the interparticle distance) is approximately the same or less than the distance of the nanoparticles, the size of

WO 01/051665

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the eligenometricies attended to them, and the sprinting of the two-newtricies where it they are photodomed in the target unclose del diffusion to core change with the otherwhen when the eligenometricies consquently en injustment hybridizars with the mentile acid targets as form aggregates. For instance, gold tomogeties with channess of CD them will be produced as the change when aggregated using a higheracteristic attended to the managements desirage the hybridizar with the produce as sold recompared to hit produce as sold recompared to the hybridizard in the aggregation, as the result demonstrate. The resultance places are sought the aggregation, as the result demonstrate. The result and has been desirable that of the other benefits of the sold office channes regarded.

This color change has been desirable that produced to a sold in and breaking of the surface places are sold of the channes that the sold in the channes of the sold office channes regarded.

This color change shortened which place and nature the course the impact of the obligate channes are sold of the places of the pold. This color change is suitable with the places of the pold composition of the color of the channes that the channel change is not the place of the color produced the color produced the color of the channel channel

Financele 9: Assays Unive Nanoparticle-Olicomaticatide Conjugates

First microllism of each probe 1 and 2 (Figure 12A) were combined to a final Termination of the high biddle (10 A) of phosphasis, by 7 3, and 1 microllism of 20 houses with was saided to be selected. While this obstacle was forcom, thereof, and for specific on a C-13 TEC/plane, a blue ceith relief and develop. The similar solution containing 12.2 and information of each produce of 23 microllism of 10 microllisms of each produce of 23 microllisms of 10 microllisms of each produce of 23 microllisms of 10 microllisms of each produce of 23 microllisms of 10 microllisms of 63 microllisms of 10 microllisms of 64 microllisms of 10 microllism

Similar experiences were personned in the presence of human saliva. A solution continuing 1.2 microllism of sandy probe 1 and 2 and 0.25 microllism of largard 3 was based to 70°C. After cooling to soon temperature, 2.5 microllism of a saliva solution (bustom saliva dilused 1:10 with userly was added. After the resultant solution was

PCT/ES01/01190

frozen, thewed and then sported onto a C-18 TLC plate, a blue spot was obtained, indicating hybridization of the probes with the surget. In control experiments with no target added, blue spots were not observed.

- Xuanquir (R. August ), Livin's (Versprendigh-Officented and Confirment
  An away was performed as informed in Figure 12A. Prol. 2, plan microscope
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- tion Monkey with processing the process of the control of the cont

DNA was attached to the surfaces by socking the modified glass slides in a 0.2 OD (1.7 ;µM) solution containing freshly purified oligenucleoticle (\*\* third)

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ATGCTCAACTCT (SEQ ID NO:335) After 12 hours of soaking time, the slides were removed and rinsed with water.

To demande the shifty of an analysis DNA cases to brist assegnation to the modified as betters. In a Single objective tools are apprecial. The licities of programs of the modified as betters. In a Single objective tools are apprecial. The licities of the same and the second of the shifty of the same and the second of the same and the second of the same and the same a

30 and cooleans to transperser pink cione. See Figure 10A.
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To verify that the oligomeclestide modified gold nanoparticles were attached to the oligomecleotide modified surface through DNA hybridization interactions with the